Universidade de Aveiro
Ano 2016

Departamento de Química

MARTINIQUE DA SILVA NUNES

ESTUDO DO EFEITO DE TRATAMENTO ENZIMÁTICO SOBRE RESISTÊNCIA FÍSICA EM TMP

STUDY OF ENZYMATIC TREATMENT EFFECTS ON PHYSICAL STRENGTH IN TMP

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Engenharia Química, realizada sob a orientação científica do Doutor Dmitry Victorovitch Evtyugin, Professor Associado com agregação do Departamento de Química da Universidade de Aveiro, de Hanne Høst Pedersen, M.Sc., e do Doutor Pedro Emanuel Garcia Loureiro, investigador no Technical Industries Application Research Department da Novozymes A/S, Dinamarca.

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Palavras-chave

Resistência física, TMP, refinação, xilanase, mananase, celulase, lacase, tração, rasgamento, rebentamento, características da fibra.

Resumo

A resistência física é essencial para a integridade do papel fabricado em qualquer uma das suas aplicações.

O objectivo deste projeto foi estudar o efeito que o tratamento de TMP por xilanases, mananases, uma celulase e uma lacase teria sobre a resistência à tração, rasgamento e rebentamento do papel fabricado a partir deste tipo de pasta.

Curvas de concentração foram efetuadas para cada uma das enzimas a testar na TMP disponível. A pasta foi previamente branqueada com H_2O_2, 3,00% (w/w) e submetida a 1000 revoluções no refinador PFI. As xilanases foram NS-51191, NS-51207 e NS-51168; as mananases foram NS-51184 e NS-51180; a celulase foi NS-51137; e a lacase foi NS-51003. As curvas de concentração foram repetidas para NS-51180 e NS-51003 de modo a verificar os resultados nos primeiros testes. Também foram medidas características da fibra, a serem comprimento, largura, percentagem de finos, "curl" e "kinks"/mm, bem como TOC.

As estratégias típicas de refinação e branqueamento aplicadas tiveram efeitos positivos, do ponto de vista estatístico, motivo pelo qual foram plicados em todos os ensaios com tratamento enzimático. Nos ensaios onde houve tratamento enzimático, verificaram-se índices de resistência fracos, capacidade de resposta reduzida ou nula por parte das enzimas e um grau não desprezável de variabilidade foram verificados durante os ensaios. A explicação para tal ocorrência foi a variabilidade inerente associada a pastas mecánicas, TMP em particular, as condições bastante uniformes de tratamento (T = 60°C, tempo de incubação igual a 60 minutos, pH ajustado conforme a enzima em estudo) e a presença significativa de lenhina, que impediu o acesso das enzimas a camadas mais internas das fibras. São necessários testes em condições de temperatura, tempo de incubação e pH diferentes para ter a certeza de que as enzimas não conseguem dar um contributo positivo no âmbito da resistência física.

Keywords

Physical strength, TMP, refining, xylanase, mannanase, cellulase, lacasse, tensile, burst, tear, fiber characteristics.

Abstract

Physical strength is essential for the integrity of the manufactured paper in any of its applications.

The aim of this project is to study the effect treatment of TMP by xylanases, mannanases, cellulase and laccase would have on the tensile, tearing and bursting strengths of paper manufactured from this type of pulp.

Dosage curves were performed with each of the enzymes to be tested on the available TMP. The pulp was previously bleached with H_2O_2, 3,00% (w/w) and submitted to 1000 revolutions in the PFI mill. Xylanases were NS-51191, NS-51207 and NS-51168; mannanases were NS-51184 and NS-51180; cellulase was NS-51137; and laccase was NS-51003. Repeatability trials were done with NS-51180 and NS-51003 to verify the results in the first tests. Fiber characteristics, i.e. length, width, percentage of fines, curl and kinks/mm, as well as TOC, were also measured.

The typical refining and bleaching strategies applied had a statistically positive effect, which is why they were applied in all enzymatic treatment trials. Whenever enzymatic treatment was performed, weak resistance indexes, reduced or no responsiveness by enzymes and a not negligible degree of variability were verified during the assays. The explanation for such occurrence was the inherent variability associated with mechanical pulps, TMP in particular, fairly uniform treatment conditions (T = 60 ° C, incubation time equal to 60 minutes, pH adjusted according to the enzyme at study) and the presence of lignin, which prevented the enzymes from accessing the inner layers of the fibers. Tests at different temperature, incubation time and pH are required to make sure that the enzymes fail to make a positive contribution in terms of the physical resistance.

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Abbreviations

A/S	Aktieselskab (stock-based corporation)
MP	Mechanical pulps
ES	Enzyme solution
P&PI	Pulp and Paper Industry
DP	Degree of polymerization
TMP	Thermomechanical pulp
RMP	Refiner mechanical pulp
EC	Enzyme Commission
odp	Oven-dry pulp
CSF	Canadian Standard Freeness
DFR	Drainage Freeness Retention
BW	Basis weight
app.	Apparent
TC	Total Carbon
IC	Inorganic Carbon
TOC	Total Organic Carbon
conc.	concentration
rep	Repetition

EDTA	Ethyldiaminetetraacetic acid
Na_2SiO_3	Sodium metasilicate
$MgSO_4$	Magnesium sulfate
NaOH	Sodium hydroxide
H_2O_2	Hydrogen peroxide

l	Length of individual fibers (mm)
b	Width of individual fibers (μm)
f	Curl of individual fibers (%)
L	Real length of individual fibers (mm)
a	Area of fines
A	Area of analyzed objects

Aim and Scope

Novozymes A/S is a company that specializes in the production of industrial enzymes. These enzymes can be implemented in various different industries to economic and environmental gains. The main sphere of action of Novozymes encompasses Household Care, Food & Beverages, Bioenergy, Agriculture & Feed, and Technical & Pharma. [1]

Forest products are one of the areas of focus of Technical & Pharma. In this department, enzymes have been developed for applications such as bleach boosting, deinking, pitch control and fiber modification.

The aim of this project is to study the effects of a selected group of these enzymes on strength properties in mechanical pulps (MP), which are known to be relatively weak in comparison to other pulp in the market, to find and follow-up on those that yield positive results. The intent is to expand the reach of Novozymes in regards to forest products. The typical end uses of this type of pulp, as well as the classes of enzymes that have been recorded to give promising results in this field, are taken into consideration.

In order to realize whether the actions of the enzymes to be studied can also improve strength properties in MP, tests are performed so as to find an adequate level of refining and enzyme solution (ES) concentration that maximize positive gains that may occur. Thus, refining and dosage curves are generated. PFI mill and xylanases, mannanases, a cellulose and a laccase are the instruments used for this purpose. Further trials are executed on those that show positive results, overall, to confirm those results and potentially study them more deeply. The chosen substrate is thermomechanical pulp, very common for newsprint, tissue paper and paperboard. A bleaching step with hydrogen peroxide (H_2O_2) are also included so as to resemble pulps destined for the aforementioned applications more closely.

Chapter 1 Background

1.1 Paper: an overview

Paper is defined as a network of fibers formed on a screen after dewatering a fibrous slurry, commonly known as pulp. It was invented in its current shape in China, around 105 AD, and spread as a means of communication, in both institutions and the masses. Industrialization starting in the 17th century, mechanization and further technological advancements have expanded its use beyond printing to hygiene, decoration, packaging, among others. This is also the reason behind its increasing demand. [2]

The importance paper has today in everyday lives makes it a commodity. As such, production costs must remain low in order to ensure it is affordable to the customer. Price is not the only aspect taken into consideration when the time to buy comes. Growing environmental and safety awareness have steered the Pulp and Paper Industry (P&PI) to comply with standards that have made it more efficient in energy and water consumption and more rigorous in treating its effluents. [2]

The same public concern with the environment that has pushed the P&PI to improve its processes has influenced the share the different sources for manufacturing paper have on the market. In the past, virgin fiber sources were preferred, i.e. chemical and mechanical pulp, but recycled fibers have gained relevance and nowadays are as procured as the virgin fiber material (Figure 1.1). [2] Herein lies one of the biggest challenges both chemical and mechanical paper mills face to continue being relevant in a world of limited resources.

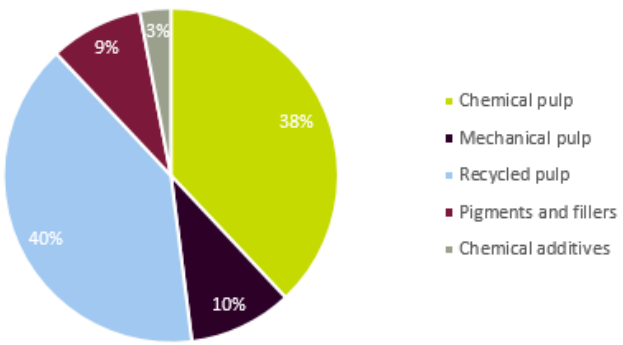

Figure 1.1 – Raw materials for paper and board production [2].

1.2 Structure and ultrastructure of wood cells

The major source for the fibers of paper is wood. Wood is categorized into two main groups: softwoods and hardwoods. Both are widely distributed around the world. Softwoods are comprised mostly of tracheids (~ 90% of wood volume), while there is more differentiation in hardwoods. [3] Table 1.1 summarizes the different types of cells and their respective function.

Table 1.1 – Main functions of the various types of cells in wood [4].

	Mechanical function	**Conducting function**	**Storing function**	**Secreting function**
Softwoods	Latewood tracheids	Earlywood tracheids	Ray parenchima	Epithelial cells
		Ray tracheids	Longitudinal parenchyma (resin canals)	
Hardwoods	Libriform fibers	Vessel	Ray parenchyma	Epithelial cells
	Fiber tracheids	Vessel tracheids	Longitudinal parenchyma (resin canals)	

As previously stated, the main cells of softwoods are tracheids. More specifically, there are earlywood (springwood) and latewood (summerwood) tracheids, which have smaller radius and thicker cell walls than the former [3].

The wall of one of these tracheids or "fibers" is comprised of several layers [5]. More specifically, each cell has a primary wall and a secondary wall, which has three layers, as defined by the orientation of the fibrils, cellulose aggregates [5]. Different continuous cells are separated by the middle lamella, very high in lignin content [5]. The structure of the cell is further elaborated upon in Table 1.2 and Figure 1.2.

Table 1.2 – Layers of softwood tracheid (20-40 μm dia.) [5].

Middle lamella (ML)	• Bond between fibers, mostly lignin
Primary wall (P)	• A thin, relatively impermeable covering about 0,05 μm thick
Secondary wall (S)	• Makes up the bulk of the cell wall; forms three distinct layers characterized by different fibril alignment: ○ S_1 is the outer layer of the secondary wall (0,1-0,2 μm thick) ○ S_2 forms the main body of the fiber and is from 2 to 10 μm thick ○ S_3 is the inner layer of the secondary wall (~ 0,1 μm thick)
Tertiary wall (T)	• Same as S3
Lumen (L)	• The central canal of the fiber (void)

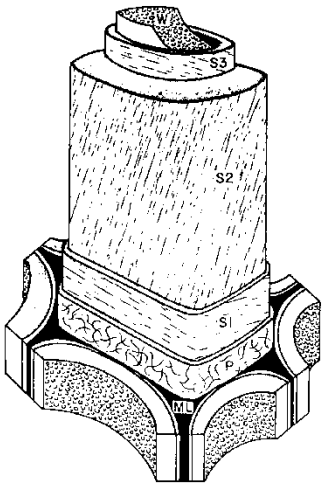

Figure 1.2 – Diagram of the cell wall [5].

On the molecular level, the main components of wood are cellulose, lignin, hemicelluloses and extractives [3]. The composition and distribution of these macromolecules vary between softwoods and hardwoods (Figure 1.4) and within the different layers of the cell wall (Figure 1.3).

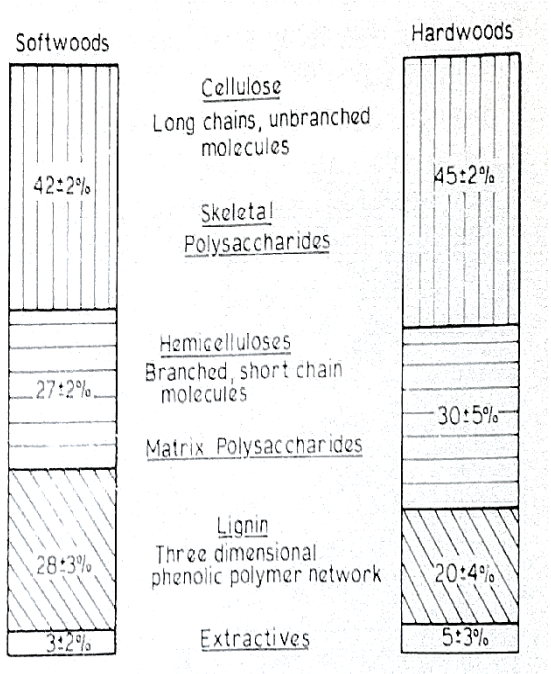

Figure 1.4 – Average composition of softwoods and hardwoods [5].

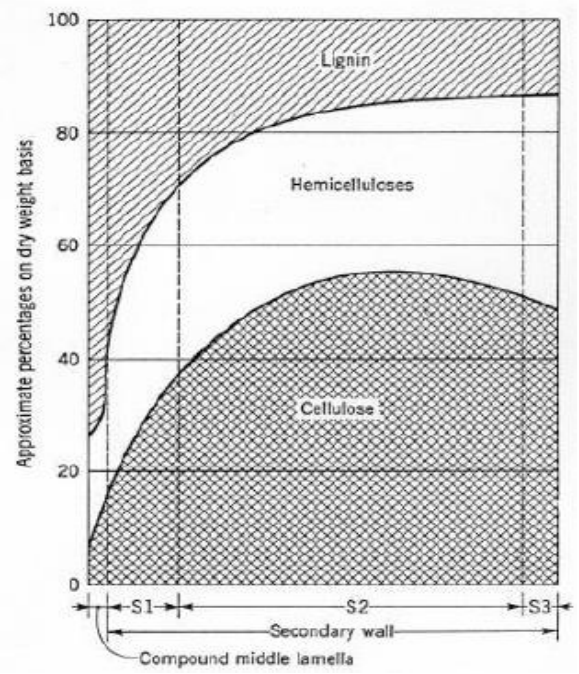

Figure 1.3 – Distribution of the main wood components across the fiber cell wall [24].

Cellulose, the most abundant polymer in plants, is a linear homopolymer of β-1,4-glycosidic linked D-glucopyranose units. The molecular structure is showcased in Figure 1.5.

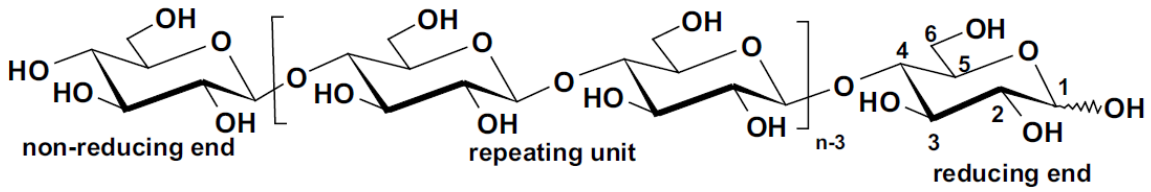

Figure 1.5 – The molecular structure of cellulose. The repeating unit is designated as cellobiose and consists of two glucopyranose units linked together (Sixta's Handbook of Pulp).

The presence of hydroxyl groups, as seen in Figure 1.5, confer cellulose the ability of forming hydrogen bonds, both within the polymeric chain and with other neighboring chains and water. Despite its hydrophilicity, cellulose does not dissolve in aqueous solutions due to its high degree of polymerization (DP). DP refers to the number of times the basic unit is repeated along the polymeric chain, $C_6H_{10}O_5$ in this case. In P&PI, the weighed-average DP is usually between 600 and 1500. [5]

The potential for intermolecular bonding is not limitless, though, since cellulose is embedded in matrix of hemicelluloses, lignin and other low molecular weight substances.

Hemicelluloses, unlike cellulose, are heteropolymers which can be constituted of hexoses (glucose, mannose, galactose) and pentoses (xylose, arabinose). These sugars, along with uronic acid, form several polymeric structures that may resemble either cellulose or lignin more closely, depending on the plant species. Their DP is much lower than that of cellulose ($50 < DP < 200$), which makes them more easily dissolved. [5]

After cellulose, lignin is the most abundant polymer in plants. An amorphous, highly polymerized substance, lignin is formed by phenyl propane units linked together in three dimensions. Its main role is to form the middle lamella, which cements the fibers together. [5]

Extractives are wood components that are soluble in neutral organic solvents (lipophilic) or water (hydrophilic) [6]. They are almost exclusively composed of extracellular and low-molecular-weight compounds [6]. Although usually present in minor fractions, they comprise a vast number of individual compounds [6]. Fatty acids and their esters are the major constituents of wood extractives in most wood species [7].

1.3 Pulping

The vast majority of paper is manufactured from wood of high plants. The wood, in turn, must undergo a series of stages prior to the generation of sheets, namely the reduction to its basic units, the fibers. This is also known as pulping.

Pulping can be realized by chemical or mechanical processes. Chemical processes rely in the combined action of chemical and energy agents, i.e. heat, to soften and remove the lignin by dissolving it from the source, followed by mechanical refining to individualize the fibers in a step called

defibration. Mechanical processes forgo the addition of chemicals in favor of abrasive refining between discs or grinding. The main processes under this category are represented in Figure 1.6. Depending on the end use, the resulting unbleached pulp may be more extensively treated by going through screening, washing, bleaching and purification. [8]

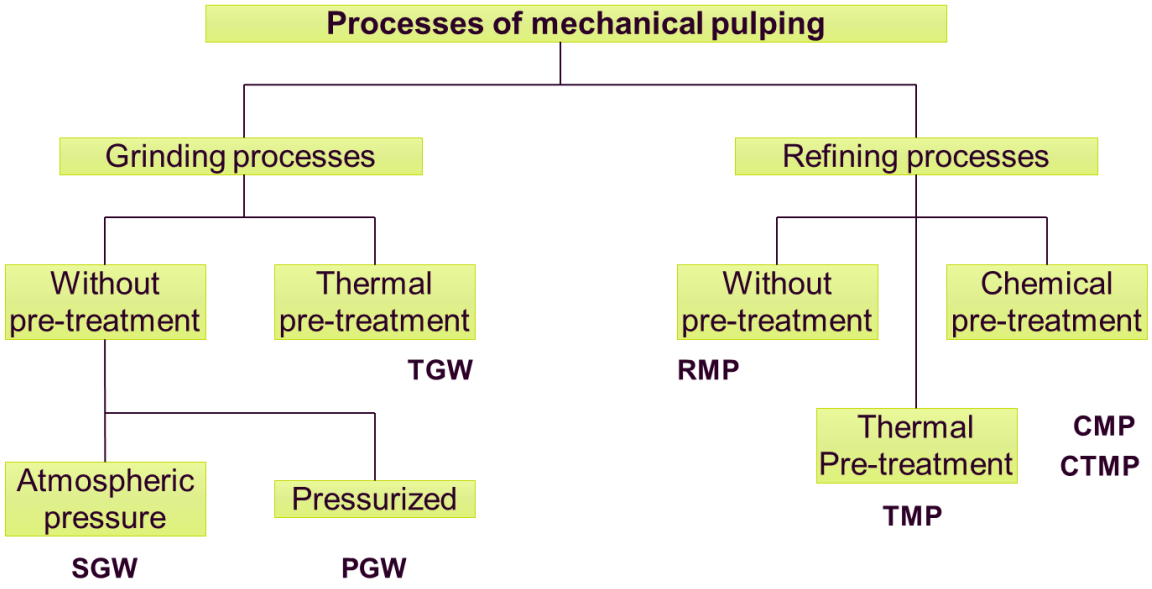

Figure 1.6 – Overview of the main mechanical processes for pulping [8].

Thermomechanical pulp (TMP) is the most prominent of the pulps derived from refining processes. It is a modification of the refiner mechanical pulp (RMP) as chips undergo a pre-treatment with steam at temperatures that range between 110°C and 130°C for 2 to 5 minutes. Refining may occur in one or two stages, usually at high pressures [5]. Screening, cleaning and brightening are carried out in a similar fashion to the other mechanical processes [5].

Due to the importance it has for the outcome in MP, refining is elaborated upon right after.

1.3.1 Refining

As previously stated, MP are obtained either through grinding of wood logs by a grinding stone or refining of wood chips by a two-disc refiner.

Refining developed as a means to address the shortcomings of grinding: by working with wood chips instead of wood logs, matter became much easier to handle [5]. Because of this, output of production volumes became larger and the processes became more automated, which lead to a decrease in labor costs [5]. Moreover, the finished pulps had a higher content of long fibers and turned out to be stronger [5].

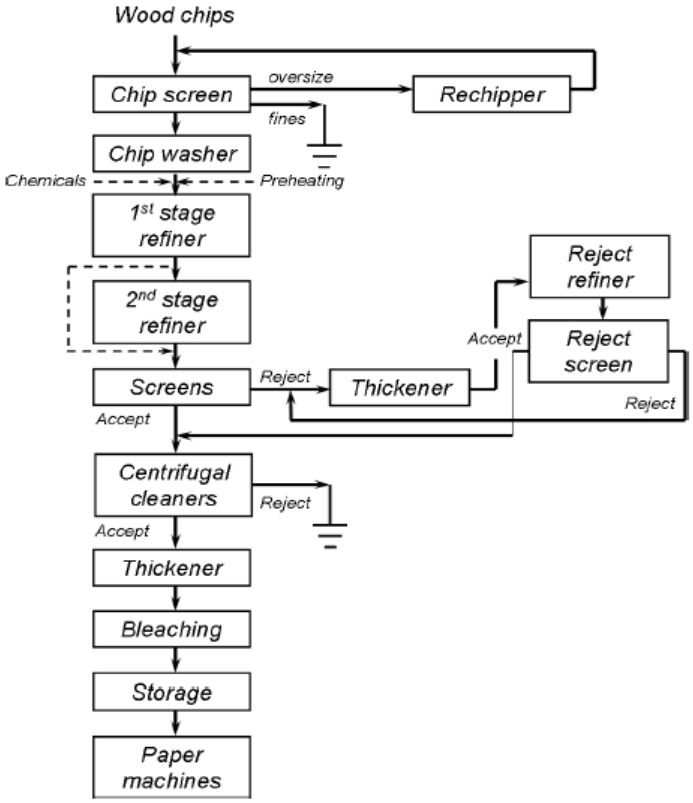

Figure 1.7 – Typical flow sheet of a modern refining [8].

A simplified representation of typical refining processes can be consulted in Figure 1.7.

The aim of refining, as with grinding, is defibration, i.e. delivering fibers from their wood source while also separating them from one another. This step can be regarded as the sequence of the following actions:

1. Reduction of the wood chips to matches.
2. Reduction of those matches to fibers.
    a. Stress by pressure pulsation and shearing of the fiber – fatigue of the middle lamella.
    b. Thermal softening of lignin and hemicellulose.
    c. Latency, i.e. agglomeration of delivered fibers.
    d. Fiber cutting.

3. Fibrillation of the delivered fibers and fiber bundles.

The two underlying phenomena for a successful defibration are:

1. Deformation of the fibers;
2. Softening of lignin.

1.3.1.1 Deformation of the fibers

Thanks to the hydroxyl groups, cellulose can form hydrogen bonds both intramolecularly and intermolecularly. During defibration, fibers are subjected to effects of high-frequency compression and decompression at the interface between the wood and the grooves and bars of the discs. These frequencies manage to break the bonds between fibers as well as bonds within the fibers, thus deforming them. The major changes the fibers undergo are listed as fibrillation, fines formation and fiber shortening [9]. How these changes reflect upon the physical strength of the resulting paper depends on the extent to which each of these changes were verified.

1.3.1.1.1 Fibrillation

Fibrillation occurs when mechanical action produces rough surfaces on fibers [10]. Two types of fibrillation have been observed during refining: internal and external fibrillation.

Internal fibrillation alludes to the delamination of the P and S_1 layers, caused by the compression and decompression effects taking place inside the refiner. Consequences of this type of fibrillation include swelling, as a result of bonds between cellulose and hemicelluloses, cellulose and lignin, and hemicelluloses and lignin, as well as bonds between cellulose fibrils, breaking apart and thus expanding the pore structure inside the cell wall; and increase of flexibility/conformability (collapsibility). These factors are positive because closer contact between fibers is promoted, and bonding becomes stronger. [9]

External fibrillation alludes to the pilling off of fibrils from the fiber surface after exposure of the S_2 layer. The main benefit of this effect is the increase of the specific surface area of fibrils. There are also some hydrophilic compounds from the cell wall that are released, forming gelatinous layers that also help to improve bonding between fibers.

The very fine fibrous material that is released during external fibrillation is known as crill (Figure 1.8). These particles are 0,25 µm in width and approximately 100 times thinner than fibers [11]. This material can amount to 50% of the total free surface area, despite being about 1% of the mass of the furnish [11]. The more crill that can be found on and around the fibers, the stronger is expected to be (Figure 1.9).

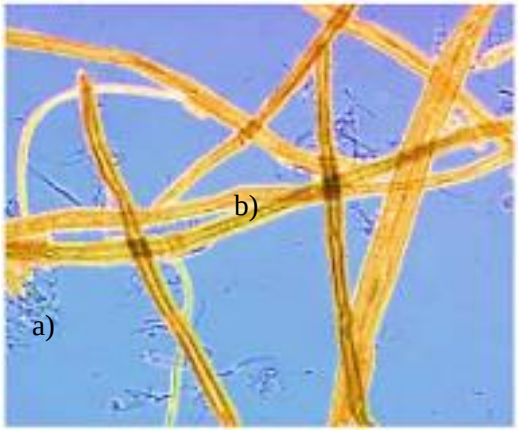

Figure 1.8 – Still of cellulosic fibers. Crill (a) is the very fine material attached or detached from the fibers (b) [11].

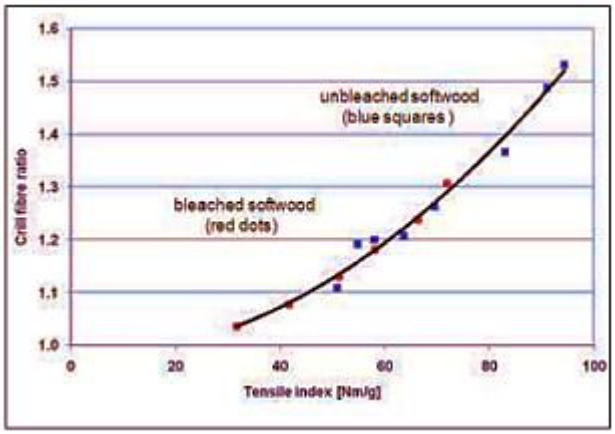

Figure 1.9 – Relation between crill and tensile strength index, as measured at Innventia [11].

1.3.1.1.2 Fines formation

Fines are essentially materials that can go through a 76 µm (200 mesh) screen [10]. These include pulp fines, which come from parenchyma and ray cells and are present in unbeaten pulp and can also be generated during refining, as a result of external fibrillation or fiber shortening. These fines consist of fragments of the P and S layers [9]. Fines are known to have a high surface area, typically five times that of fibers per unit of weight, but it can be as high as ten times. Fines are also known to absorb water and swell more than fibers. They have the negative effect on drainage time, though. Typical values for fines in newsprint are in the range between 40% and 60% [10].

1.3.1.1.3 Fiber shortening

Unlike the previous two effects, reduction of the fiber length, usually estimated as a statistical average that may be numerical or arithmetic, length-weighted and weight-weighted, tends to be detrimental to physical strength.

Shortening happens by the action of direct shearing forces exerted by the refiner bars on the pulp or by failure from pulling from a network of fibers they might be bonded to. [9]

1.3.1.2 Softening of the lignin

Defibration can also be achieved by softening the lignin in the middle lamella and primary wall, which binds fibers together, by mechanical, thermal or chemical means. Mechanical softening of lignin happens in concert with fiber deformation during refining. Thermal softening, on the other hand, not only happens during refining, but also before this step, in the processes that include thermal

pretreatment. Chemical softening, which is beyond the scope of this project, involves a gentle treatment combined with the refining step. [8]

Temperature has a very important role in the development of the properties of the mechanical pulp produced. At the defibration stage, care must be taken that the temperature increase due to friction of metal against wood as well as friction of wood against wood is close enough to the temperature of softening of lignin so as to ensure defibration with minimal damage to the fibers. The best-case scenario would be defibration occurring at slightly greater temperature than that of the softening of the lignin. In this situation, the middle lamella and primary wall are removed and milled down to fines, giving way to the secondary wall (S1) to be fibrillated without damaging the fibers. It is estimated that lignin of moist chips softens at 120-135°C. Defibration temperatures aim to be in the range between 100°C and 130°C. Reaching higher temperatures, namely greater than 140°C, would result in a harsh and coarse, unfit-for-papermaking pulp in spite of the well-softened lignin facilitating the deliverance of fibers at minimal mechanical energy expenditure. Moreover, once temperature decreases and lignin hardens as a result on the fiber surface, resistance to fibrillation increases significantly. Lower temperatures would also output coarse pulp with low strength properties. Thus, the better temperature ranges are the ones where a better compromise among all the effects at play is reached. [5], [8]

1.3.1.3 Quality factors

The aforementioned phenomena depend on the coalition of several parameters. Some are related to the raw material, such as the wood chip quality, others to the characteristics of the refiner, for example its design and the associated intensity of the refining, but most are process-related: temperature, pressure and duration of thermal pretreatment, addition of chemicals and the consistency in first-stage refining [8]. These factors influence the energy demand of the process, as measured by the specific energy consumption and its distribution between refining stages. This parameter is most linked to the quality of the produced pulp. However, it is difficult to measure the specific energy consumption, so freeness is used as a control parameter instead. Usually, the greater the energy expenditure, the lower the freeness. Some caution is required, though, because the relationship between these two variables is deeply informed by the quality of the wood raw material and, consequently, wide variation may be verified [5].

1.3.1.4 Outcome

The main asset of MP is their competitive price, for which the yield, ranging from 85% to 95%, is responsible. In addition, MP are on the basis of highly opaque bulky paper well-suited for printing. In consequence, MP are primarily used for newsprint. Nevertheless, MP is very demanding energy-wise; the production a metric ton of paper may require the input of 1.3 to 3.0 MWh, depending on the end use and the process involved. Moreover, sheets discolor easily when exposed to light

and are usually weak and cannot withstand the forces in high-speed newsprint machines on their own [5]. The standard strategy to address this issue has been the addition of some chemical pulp furnish. While TMP managed to reduce the amount of necessary chemical pulp from the typical 25% that would be mixed with groundwood, this issue hasn't been completely solved yet [5]. These pulps are most often produced from softwoods due to their long fibers [5].

Other applications for MP are tissue paper and paper boards.

1.4 Physical strength of paper

In order for paper sheets to be feasible, there are two core requirements that must be fulfilled:

1. Fiber conformability, i.e. the ability to be matted into a uniform sheet.
2. Strong inter-fiber bonding.

The degree to which both requirements are met is deeply linked to factors related to wood source and its chemical composition and distribution, and basic properties of fibers, as well as their physical and chemical integrity.

Regarding the structure of fibers, both the length and the cell wall thickness must be taken into account. There must be a minimum length for inter-fiber bonding to be possible. Moreover, length is virtually proportional to tear strength. The most notable role of cell wall thickness has to do with inter-fiber bonding. Thin-walled cells are more conformable than thick-walled ones, since they collapse more easily. [5]

1.4.1 Physical strength properties

There are several parameters that can be measured to assess the physical strength of paper. Selecting the appropriate ones to measure depends on the application the paper is destined to.

Tensile strength corresponds to the maximum stress a specimen can withstand by being stretched under prescribed conditions before failing and rupturing. It is usually expressed as a force per unit width of the specimen, $N \cdot m^{-1}$. [12]

Bursting strength is also relevant for characterizing packaging papers. It is a measurement of the resistance paper is able to offer to a force exerted on one of its sides until it fails and ruptures by a specified instrument, under prescribed conditions. [12]

Tearing resistance alludes to the force required to tear a specimen under standardized conditions. [12]

The aforementioned strength properties are related to the fibers in terms of both their individual strength and their capacity to bond with neighbor fibers. It is known that drying pulp before papermaking induces significant changes because irreversible internal bonds are established. The fiber is made stiffer and stronger internally as it loses some of its ability to swell and bond with other fibers. Thus, the paper becomes more resistant to tearing, but weaker in tensile and bursting strengths. It is inferred from this situation that tear resistance is related to the inherent physical integrity of the fibers, while tensile and bursting strengths are deeply linked to inter-fiber bonding. [5]

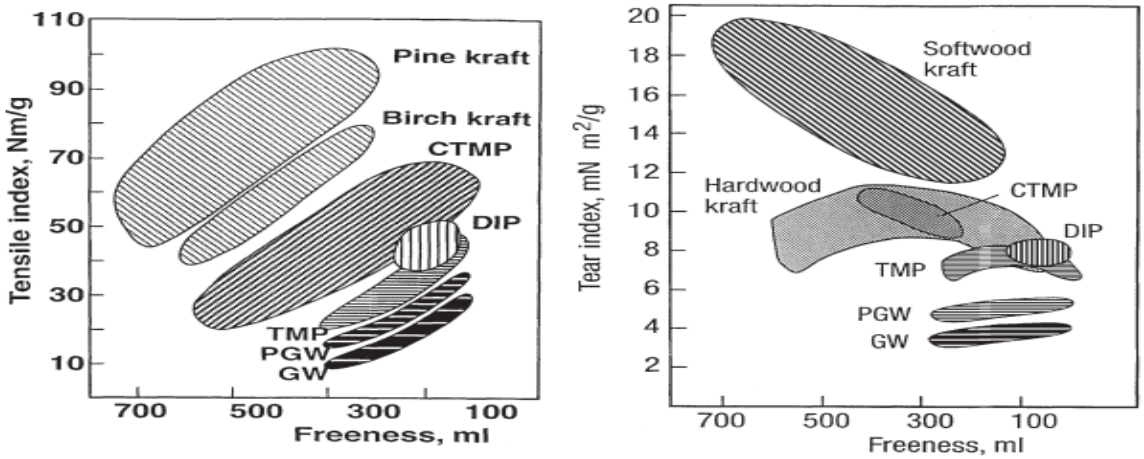

Figure 1.11 – Tensile and tear strength indexes of different pulps depending on freeness [13].

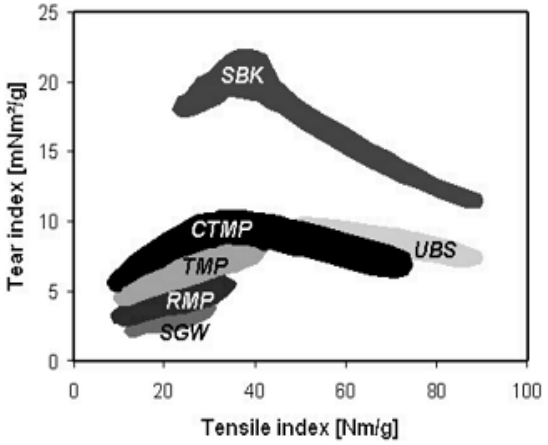

Figure 1.10 – Tear strength index versus tensile strength index of different pulps [13].

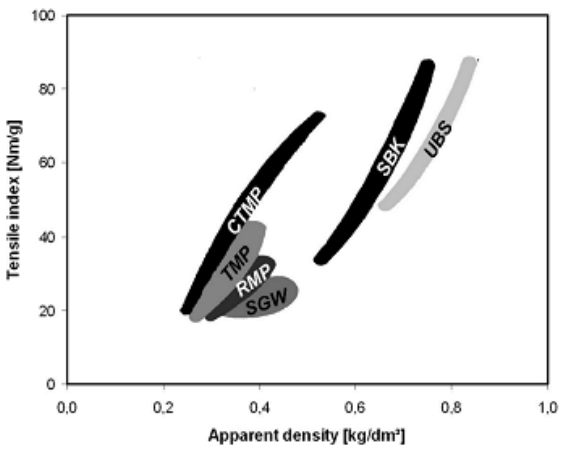

Figure 1.12 – Tensile strength index of different pulps depending on apparent density [13].

The plots represented in Figure 1.11, Figure 1.10 and Figure 1.12 show typical values of physical strength indexes in comparison to other properties for different pulps, TMP being the one in focus in this project. For values of freeness ranging from 60 to 300 mL, tensile strength indexes between 15 and 45 Nm/g are to be expected, and for a range of freeness between 70 and 270 mL, tear strength indexes between 6 and 8 mNm^2/g are to be expected (Figure 1.11). When tensile strength

indexes are between 14 and 45 Nm/g, tear strength indexes between 4 and 9 mNm^2/g are to be expected (Figure 1.12). For apparent density values between 0,25 and 0,45 kg/dm^3, values of tensile strength indexes between 15 and 45 Nm/g (Figure 1.10). [13]

1.5 Enzymes

Enzymes are homogeneous biological catalysts. These compounds, ever present in biological systems, are primarily proteins, although catalytically active RNA molecules have also been identified. Enzymes have an active site that binds the substrates, the reactants, and processes them into products through an alternative reaction pathway to the uncatalyzed one, with lower activation energy, thus bringing the reaction to equilibrium at rate that can be up to 10^6 times faster (Figure 1.13). The active site returns to its original state after releasing the products. Enzymes do not form or degrade by participating in the reaction they're catalyzing and they are in the same phase as the reaction mixture. [14]

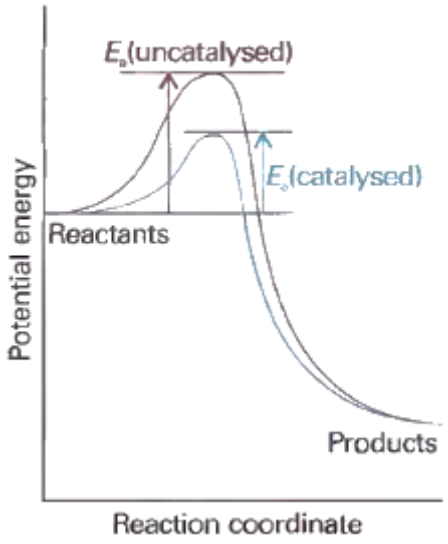

Figure 1.13 – Comparison of activation energy between an uncatalyzed reaction and the same reaction, but catalyzed [14].

In addition to the catalytic effect, enzymes are known to be very specific. This is because of how the active site binds to the substrate. Groups in the substrate interact with groups in the active site and form intermolecular bonds, namely hydrogen bonds, electrostatic, or van der Waals interactions. There are two models that explain this process. The lock-and-key model, substrate and active site have complementary 3D structures that dock perfectly into each other without the need for major atomic arrangements. The induced fit model, empirically favored, suggests there's a conformational change in the structure of the active site when it binds to the substrate (Figure 1.14). [14] Depending

on the reaction they catalyze, enzymes can be classified into six classes, according to the Enzyme Commission (EC) (Table 1.3) [15], [16].

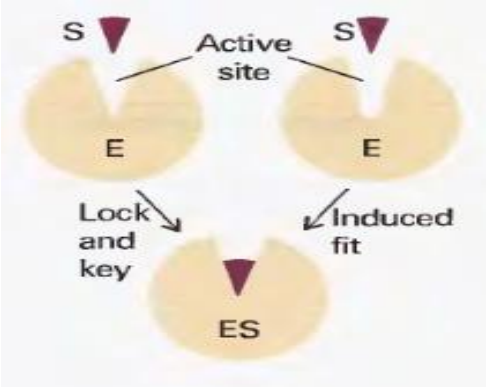

Figure 1.14 – Forming of the enzyme-substrate complex: lock-and-key and induced fit models [14].

Table 1.3 – Classification of enzymes and their reactions [16].

Class	Reaction	Industrial enzymes
EC 1. Oxidoreductases	Oxidation-reduction reactions.	Catalase, glucose oxidase, laccase
EC 2. Transferases	Transfer of various groups from one molecule to another.	Glucosyltransferase
EC 3. Hydrolases	Hydrolysis, cleavage of substrates by water.	Amylase, cellulose, lipase, mannanase, pectinase, xylanase, pectinase, phytase, protease, pullulanase
EC 4. Lyases	Addition of groups to double bonds or the formation of double bonds by elimination.	Pectate lyase, α-acetolactase decarboxylase
EC 5. Isomerases	Rearrangement of atoms within the same molecule.	Glucose isomerase
EC 6. Ligases	Joining of two molecules with covalent bonds.	Not used at present

There are several factors governing the catalytic activity of enzymes. Reactions catalyzed by enzymes are temperature-dependent, exhibiting an optimum that arises from the tension between the thermodynamic increase of reaction rate (1 in Figure 1.15) and the thermal denaturation the enzyme undergoes in high temperatures (2 in Figure 1.15). Enzyme activity also depends of the pH of the reaction mixture. All enzymes have a pH range under which they can operate (Figure 1.16). This range is influenced by factors such as ionic strength, type of buffer, temperature, substrate and coenzyme concentrations. [15]

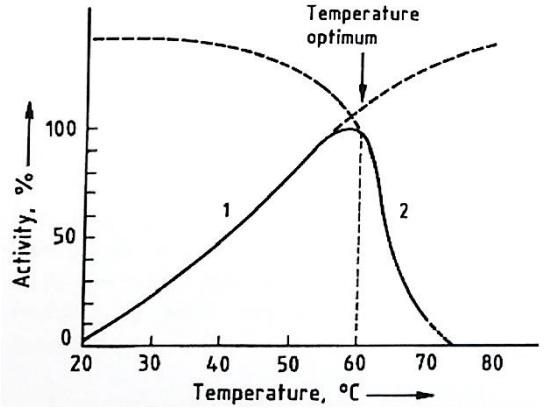

Figure 1.15 – Temperature optimum of enzyme activity. It generally occurs between 40 and 60°C [15].

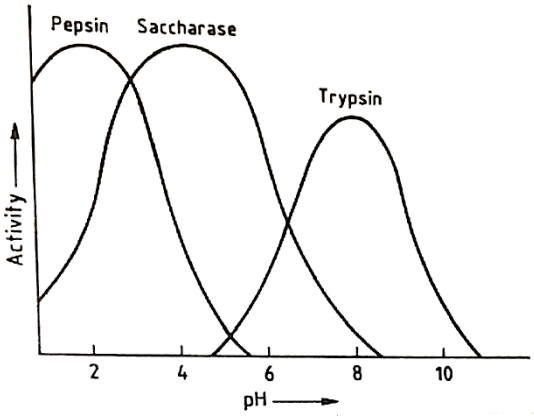

Figure 1.16 – Activity of various enzymes as a function of pH [15].

Both the catalytic activity and the specificity are the reason many different enzymes have been adapted to multiple applications in different fields since the 1800's, time when the first industrial application was successful [17]. Moreover, these traits allow industries to veer in the direction of more environmentally-friendly processes, because they help decrease, even replace, the use of harsh chemicals under milder operating conditions. Enzymes are also biodegradable, thus posing no problem to the environment. They don't pose health risks except when inhaled, for which some precautions must be taken to avoid the development of allergies. [17]

Their introduction into the market entails overcoming a few challenges, though. Enzyme production requires a high capital investment, which reflects later on in the price of the final product. Adaptations of already running processes may as well be necessary for enzyme activity to be feasible. Thus, the successful implementation of enzymes in any industry depends on its ability to provide clear economic benefits at a reasonable price and in large quantities, without affecting negatively the quality of the final product.

The value of the enzyme market in 2014 has been estimated to be $ 4,4 billion. There are several factors that make the precise sizing of the enzyme market difficult, such as scarcity of data, internal consumption and the currency in which data is reported. Having said that, the market is estimated to have grown between 5 and 10% each year from 2004 to 2014. This growth has been largely influenced by the rising price of crude oil, which steers efforts in developing alternative materials. Some of the technical industries have greatly benefited from this development. Together, they make up for the most important segment of the enzyme market (Figure 1.17). The main examples of this category are the detergent, starch, textile, alcohol fuel, leather and pulp and paper industries. Current figures for overall growth estimate it at 4 to 5% annually. [17]

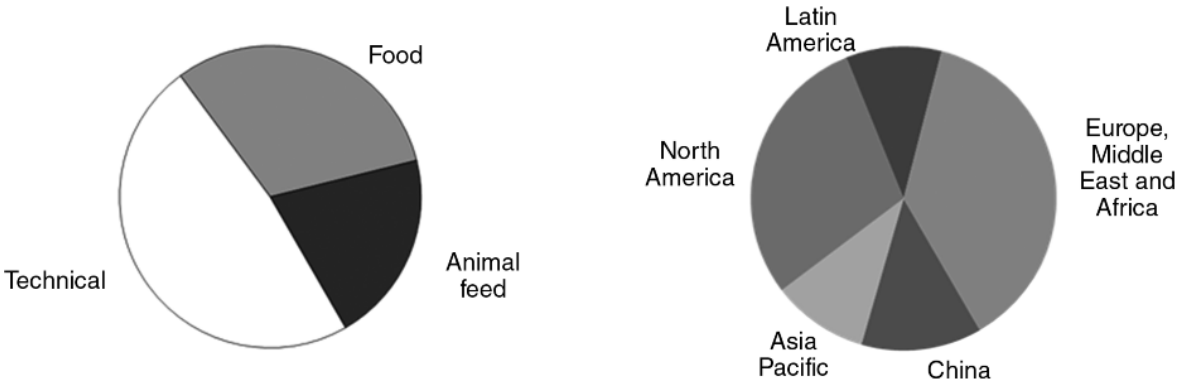

Figure 1.17 - Segmentation of the industrial enzyme market [17].

Specifically in the P&PI, the applications of enzymes have expanded considerably since xylanases were first used to boost bleachability in the 80's. This is possible because of the complex chemistry associated with wood materials, making different approaches for modification and/or improvement possible. Nowadays, enzymes are mostly used in bleaching, deinking, improvement of paper-machine runnability by hydrolysis of extractives or enhanced drainage. Recent efforts have focused in fiber modification, with the aim of modifying the performance of specific fibers in paper, tissue and board manufacture. [17] The main applications of enzymes in the P&PI are summarized in Table 1.4.

Table 1.4 – Overview of the current enzyme application areas in the P&PI [17].

Segment	Application	Enzyme class
pulp (e.g. for tissue, towel paper, and board manufacture)	bleach boosting	xylanase
	deinking	amylase, cellulase, lipase
	strengthening & refining	cellulase, laccase, hemicellulase
	drainage/dewatering	cellulase, hemicellulase
	vessel element reduction	cellulase, hemicellulase
process & equipment	starch modification	amylase
	pitch control	lipase
	stickies control	lipase
	cleaning	protease, lipase, amylase
process & wastewater	cationic demand reduction	pectinase
	color/odor removal	laccase, peroxidase
	residual management	multiple enzymes

In regards to fiber modification, several theories have been proposed to explain the underlying mechanism for enzyme intervention. The most accepted suggestion proposes fibrils and fiber bundles are attacked on the surface, peeling off subsequent layers and eventually disintegrating fibers. This can lead to increased freeness and enhanced fiber flexibility, resulting in denser paper sheets, but also to lower yields and loss in fiber strength. [18]

The enzymes that concern strengthening and refining are subsequently discussed.

1.5.1 Hydrolases

One of the six main classes of enzymes under the EC Nomenclature, hydrolases are characterized for accelerating the cleavage of substrate with the addition of water, the definition of hydrolysis. Cellulases and hemicellulases fit this category.

1.5.1.1 Celullases

As the name suggests, cellulose is the substrate of cellulases. Due to the context around this polymer, as previously discussed, it is a challenging substrate to handle.

Thus, the efficient degradation of cellulose requires the action of different cellulases, either in sequence or in concert. Traditionally, cellulases are classified as endoglucanases or exoglucanases. Endoglucanases cleave the polymer chain internally, whereas exoglucanases yield cellobiose, since it degrades the chain from its ends inward. For the most part, the former can also be linked to action in the amorphous sections of the fibers and the latter to the crystalline ones.

1.5.1.2 Hemicellulases

Hemicellulases account for all the enzymes which degrade hemicelluloses. The two most common of these molecules in wood are xylans and glucomannans, whose composition and structure vary between hardwoods and softwoods [15].

1.5.1.2.1 Xylanases

The most extensively researched hemicellulases, xylanases have been shown to be able to hydrolyze different types of xylans, which widen the spectrum of subtrates they can act upon. The most important conditions to account for in their implementation is the pH and temperature. Within their viability range for these two parameters, xylanases are known to be very active and stable. More specifically, they are classified as endoxylanases, since they cleave β-D-1,4-xylosidic linkages in xylans randomly. [15]

1.5.1.2.2 Mannanases

Reportedly with a more heterogeneous group than xylanases, mannanases catalyze the random cleavage of β-D-1,4-mannopyranosyl linkages within the main chain of mannans and other polysaccharides consisting mainly of mannose, such as glucomannans, galactomannans and galactoglucomannans. The yield of glucomannans depends on the degree of substitution and the distribution of the substituents. It is also influenced by the glucose/mannan ratio. Some manananases can hydrolyze the bond between mannose and glucose units in addition to the β-D-1,4-bond between two mannose units. [15]

1.5.2 Oxidoreductases

Also one of the six main categories of enzymes, oxidoreductases are known for catalyzing the transfer of electrons. Because radicalization is a major mechanism in the chemistry of lignin, oxidoreductases are deeply associated with lignin degradation in P&PI. However, the complexity of

such mechanisms in addition to the widely variant structure of lignin among wood species demands more investigation for a deeper understanding. The most extensively studied oxidoreductases as of recent are manganese-dependent peroxidases and laccases. The latter is further elaborated upon right after. [15]

1.5.2.1 Laccases

Laccases typically oxidize a phenolic group, which results in the reduction of O_2 to H_2O. This leads to the loss of an electron in the substrate, thus forming a free radical. From there, two pathways are available: further laccase-catalyzed oxidation or nonenzymatic reactions, e.g. hydration and polymerization. Laccases are able to work on a broad spectrum of substrates, di- and polyphenols, aromatic amines, among other compounds. [15]

1.5.3 Enzymes in mechanical pulping

As previously stated, MP have a yield up to 95%, which confers them a specific set of traits, low strength in comparison to chemical pulps being one of the most significant drawbacks. Moreover, this high yield presupposes a low accessibility of enzymes to the fibers due to structural issues, which limits the extent to which the latter can be modified. Consequently, enzymes can only act upon the pulp surface and dissolved and colloidal material solubilized in process water. Treatments of mechanical pulp by laccase were shown to reduce significantly both lipophilic and hydrophilic extractive content, although decrease of brightness has been reported as well [7]. The main courses of investigation regarding this type of pulping have been biomechanical pulping, microbial reduction of pitch components prior to pulping and enzyme-assisted refining of coarse fibers. [7], [17], [19]

Enzyme-assisted refining can be expected to work best only after primary refining. A process concept for TMP based on the treatment of the fibers with monocomponent cellulases has been developed. Research has shown that pure endoglucanases alone do not give rise to high levels of hydrolysis and that their action was necessary to improve pulp freeness [18]. Endoglucanases also decrease the integrity of the fibers and making them more collapsible under physical pressure during papermaking [18]. Care must be taken, though, because the effects of some endoglucanases are too destructive to the fibers for there to be positive effects in terms of strength properties. In the case of multicomponent cellulolytic systems, the verified trend has been that of dramatic viscosity reduction and consequent strength reduction [17]. Research has also shown xylanases preserve the strength of fibers by selectively attacking xylan and leaving cellulose relatively intact [18]. Xylanases have the ability to enhance density and increase fiber-fiber bonding [18].

Fiber modification has been enthusiastically pursued in the tissue and towel sectors, due to perceived improvements in the quality of the end products. This line of research is now pervading into the manufacture of packaging/board grades and also of printing and writing paper grades. [17]

Chapter 2 Materials and methods

The aim of this chapter is to describe the materials and methods used in this project. First, the raw materials used are referenced. Then, the methods at the base of the experiments performed in this project are described in detail. Finally, the focus shifts to detailing those specific experiments, their conditions and the potential modifications they required from the methods at their core, previously described.

The experiments referred hereafter were performed at the Technical Applications department premises in Novozymes, in Bagsværd, Denmark.

2.1 Raw materials

Softwood TMP accepts and rejects were procured from an undisclosed pulp mill in the United States of America. Raw materials were delivered at Novozymes in January 2016. Species of origin are unknown.

2.2 Methods

2.2.1 Bleaching with hydrogen peroxide

Pulp and water were mixed in small stomacher bags and pre-heated for 5 minutes in a water bath at 60°C. Solutions of the compounds in Table 2.1 were added to the pulp and mixed manually for bleaching to occur at a 12,5% consistency. Compounds were added in the order purported in Table 2.1 to minimize H_2O_2 decomposition. Concentrations in the same table were chosen after consulting Reference [20]. The stomacher bags were sealed and reintroduced into the water bath. Reaction time was 60 minutes.

Table 2.1 – Compounds added to the pulp for bleaching at 12,5% consistency [20].

Compound	Volume (µL)	Concentration (% (w/w))
EDTA	5000,0	3,00
Na_2SiO_3	5000,0	2,50
$MgSO_4$	5000,0	3,00
NaOH	5000,0	0,08
H_2O_2	5000,0	0,20

After the reaction time was up, samples were taken out of the water bath and drained into a 1,0 L Erlenmeyer with the aid of a Büchner funnel and vacuum. The pulp was washed four times

with deionized water and stored for subsequent trials. Residual H_2O_2 and pH were measured on the filtrates collected during draining, before washing.

2.2.2 Handsheet preparation

Small amounts of pulp were taken to determine the percentage of oven-dry pulp (odp), by comparison between the mass of the sample before and after drying at 160°C. Once the percentage was known, 24,0 g odp were weighed. A 2000,0 g pulp suspension was subsequently prepared by adding mixed water to the pulp while weighing. Mixed water pertains to the mixture of distilled and tap water in a 2:1 proportion, so as to minimize potential effects of tap water, known for its hardness. The suspension was disintegrated at 8000 revolutions (Figure 2.2 a) and drained twice with the use of a mesh and a Büchner funnel, so as to minimize loss of pulp in this step. The recovered pulp was transferred to beakers and mixed with filtrate or Britton-Robinson buffer, if the latter was required. The goal was to prepare a 2% consistency suspension, i.e., its mass would amount to 1200,0 g during incubation. The beakers were placed into a water bath (Figure 2.2 b) set at a predefined temperature and preheated for a fixed period of time. Suspensions were stirred at 250 revolutions per minute.

Preheating, which are referred to as pre-incubation henceforth, was followed by incubation. Duration of this step was established by the author and is related to the conditions of given trials. This topic is addressed later on in this chapter. After incubation, the beaker was removed from the water bath and the suspension drained, as previously described. Recovered pulp was mixed with filtrate to prepare a 10% consistency sample for beating, according to TAPPI Standard T 248 sp-00. In other words, the mass equaled to 240,0 g. The sample was then uniformly distributed around the wall of the stator of the PFI mill (Figure 2.1) and refined at a fixed number of revolutions. Small amounts of the filtrate were saved for pH and TOC measurements, to be referenced later in this chapter.

Figure 2.1 – PFI mill for refining on a laboratory scale.

220,0 g of the beat pulp were then used to prepare a 2000,0 g suspension to be also disintegrated at 8000 revolutions (Figure 2.2 a). Afterwards, the suspension was added to a proportioner

alongside 9000,0 g more of mixed water. The goal was to have a 2,0 g/L suspension for handsheet preparation, according to TAPPI Standard T 205 sp-95. This suspension is the basis for handsheet preparation, as well as freeness and fiber characteristics measurements, to be described later on.

A stack of either 5 or 7 handsheets was thus prepared from this suspension. For each of them, a volume between 600 and 1000 mL of the suspension was used. The reason for this apparent variability is addressed accordingly. This volume was poured into the handsheet former (Figure 2.2 c), which was then filled with distilled water, agitated and drained. The stock pulp was retained onto a mesh. Sheets of blotter paper were placed on top of the pulp and couched to ensure the integrity of the resulting handsheet. A metal plate was then placed on the handsheet, so as to be able to pile all the handsheets made into a stack. The stack was pressed twice (Figure 2.2 d), for 5 minutes and 30 seconds, and 2 minutes and 30 seconds, respectively, at 0,4 MPa. The sheets were stacked individually between metal plates and rings (Figure 2.2 e) and taken to a room acclimatized at 23°C and 50% relative humidity for testing. The stack was then left to dry for a minimum period of 24 hours before testing took place.

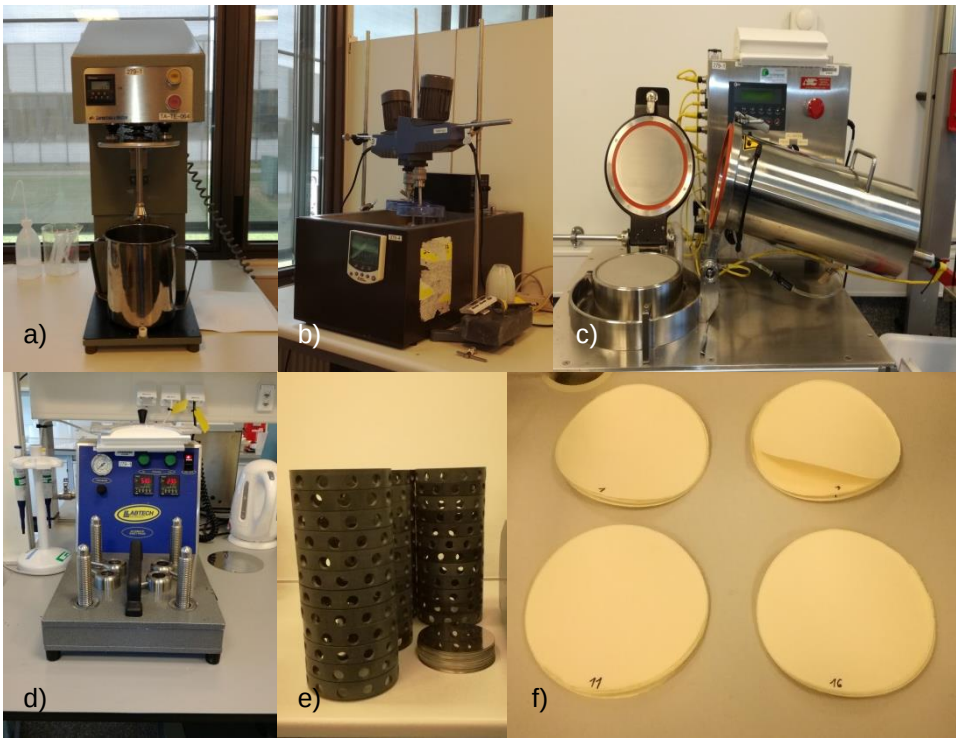

Figure 2.2 – Equipment used for preparing handsheets: a) disintegrator, b) water bath, c) handsheet former, d) handsheet press, e) rings and metal plates for piling up stacks to dry, f) bleached TMP handsheets from a given trial.

2.2.3 Pulp suspension testing

After the stacks were prepared, 5,0 L of the 2,0 g/L suspension were saved: 3,0 L destined to measure freeness, and 2,0 L to measure fiber characteristics in the PulpEye.

2.2.4 Freeness

Freeness is defined as the rate at which water drained from a stock suspension through a wired meshed or a perforated plate [12]. When determined by the Canadian Standard Freeness (CSF) test, figures are reported in milliliters (mL).

The test is taken according to TAPPI Standard T 227 om-94. One liter of the suspension is transferred to the chamber of the Drainage Freeness Retention (DFR-04) tester was used a time. This amount of suspension was added to the DFR-04 freeness tester and drained though a 16 mesh screen. The result was reported after 200,0 s.

Figure 2.3 – BTG's Mütek DFR-04 for measuring freeness. Results are reported as Canadian Standard Freeness (CSF).

2.2.5 Fiber analysis

There are several characteristics of the fibers that may have a sizable impact on the strength properties of the paper. It is thus important to account for any changes that might happen during the experiments.

The PulpEye fiber analyzer is capable of measuring several of those characteristics. The results are reported as either distributions or averages after analysis of high resolution images.

Properties pertaining to this project are the following:

Fiber length (mm): PulpEye measures this parameter for fibers longer than 0,2 mm. It is computed as follows:

$$Length = \frac{\sum l \cdot l}{\sum l} \quad (1)$$

Where l stands for fiber length for every individual fiber.

Fiber width (μm): PulpEye measures this parameter for fibers longer than the defined width limit, 0,5 mm. It is computed as follows:

$$Width = \frac{\sum b \cdot l}{\sum l} \tag{2}$$

Where b stands for fiber width for every individual fiber.

Fiber curl (%): Fiber curl alludes to how the actual fibers compare to their completely straight counterparts. PulpEye measures this parameter for fibers longer than the defined length limit, 0,5 mm. It is computed as follows:

$$Curl = \frac{\sum f \cdot l}{\sum l} \tag{3}$$

Where f stands for fiber curl for every individual fiber. This parameter is defined as:

$$f = \left(\frac{L}{l} - 1\right) \times 100 \tag{4}$$

Where l is the project length of the fiber and L is the actual length of the fiber.

Fiber kinks: a kink is an unnatural bend of the fiber, one that wouldn't be present in non-processed fibers. The results are the mean value for fibers longer than a given length, typically 1,5 mm. They can be expressed as kinks/fiber or kinks/mm.

Fines (%): Fines are defined as very short pulp fibers or fiber fragments and ray cells. PulpEye measures this parameter for fibers longer than the defined length limit, 0,2 mm. It is computed as follows:

$$fines = \frac{\sum a}{\sum A} \times 100 \tag{5}$$

Where $\sum a$ stands for the total summed area of the objects defined as fines, and $\sum A$ for the total summed area for all the analyzed objects.

Crill: This parameter pertains to very small bits of fiber, sometimes still attached to the fiber surface hat are largely stripped off during refining. Crill differs from fines for being much smaller and highly hydrated, often to the point of forming gel-like layers [12].

For the crill tests, three 2000,0 g suspensions were prepared by mixing 500,0 g of the 2 g/L suspension and water. One mixture at a time was added to the crill module of the PulpEye and assessed after prompting by the software of the analyzer. Results were recorded.

To assess the remaining fiber characteristics, one 3000,0 g suspension was prepared by mixing 500,0 g of the 2 g/L suspension and water. The mixture was added to the fiber dimensions module of the PulpEye and assessed after prompting the software.

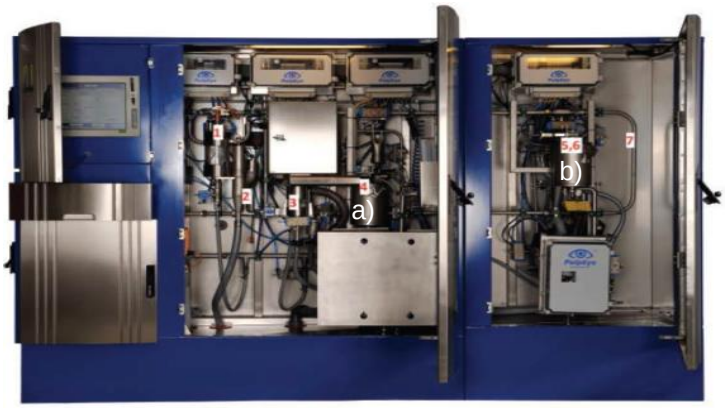

Figure 2.4 – PulpEye pulp analyzer. Modules in focus are a) crill and b) fiber characteristics [11].

2.2.6 Handsheet testing

Testing to determine basic properties, such as basis weight (BW), caliper, apparent density (app. density), ISO brightness and physical properties, such as tensile, bursting and tear strengths, all took place in a room at 23°C and 50% relative humidity.

The handsheets of the stack were removed from the rings and metal plates and were weighed individually (Figure 2.6 a). The mass was recorded in order to determine the average basis weight of the sheets.

Then, the stack was pressed together to measure thickness (Figure 2.6 b).

Afterwards, ISO brightness is measured in the ColorTouch PC Technidyne (Figure 2.6 c), according to ISO Standard 2470-2:2008. The stack is held between two bars at three different points of the stack. The numerical output is the average of the values measured in those three areas.

Preparation of the sheets for physical testing ensues, according to TAPPI Standard T 220 sp-01. The stacks are cut (Figure 2.6 d) to determine tensile strength index, burst strength index and tear strength index (Figure 2.5).

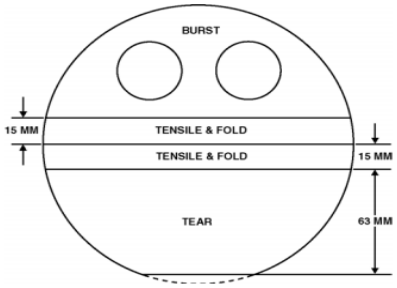

Figure 2.5 – Template for cutting handsheets for physical testing, according to TAPPI Standard T 220 sp-01.

Bursting strength (Figure 2.6 f) is measured by applying pressure on one of the sides of the sheets until it fails and ruptures, according to TAPPI Standard T403 om-97. The test is performed twice on each individual sheet, for a total of 10 values. Prior calibration of the equipment is needed by adjusting the set point to 117,9 psi.

Tear strength is measured by strapping the stack between two clamps on the Digital Elmendorf Tearing Tester (Figure 2.6 e) and releasing the pendulum to tear the stack apart after making a cut on its edge, according to TAPPI Standard T414 om-98. The test is performed along this edge. Prior to testing, the equipment is calibrated. Testing can proceed if the registered value is a fifth of the strength the pendulum can exert, with a 5% tolerance. A 800 cN pendulum was used throughout this project.

Tensile strength is tested in the INSTRON 5564 electromechanical load frame (Figure 2.6 g), according to TAPPI Standard T494 om-96. Strips of paper are clamped and stretched until the strip fails and ruptures. The test starts with a 100-mm gap between the clamps. This test is repeated twice for each stack, for a total of 10 measurements.

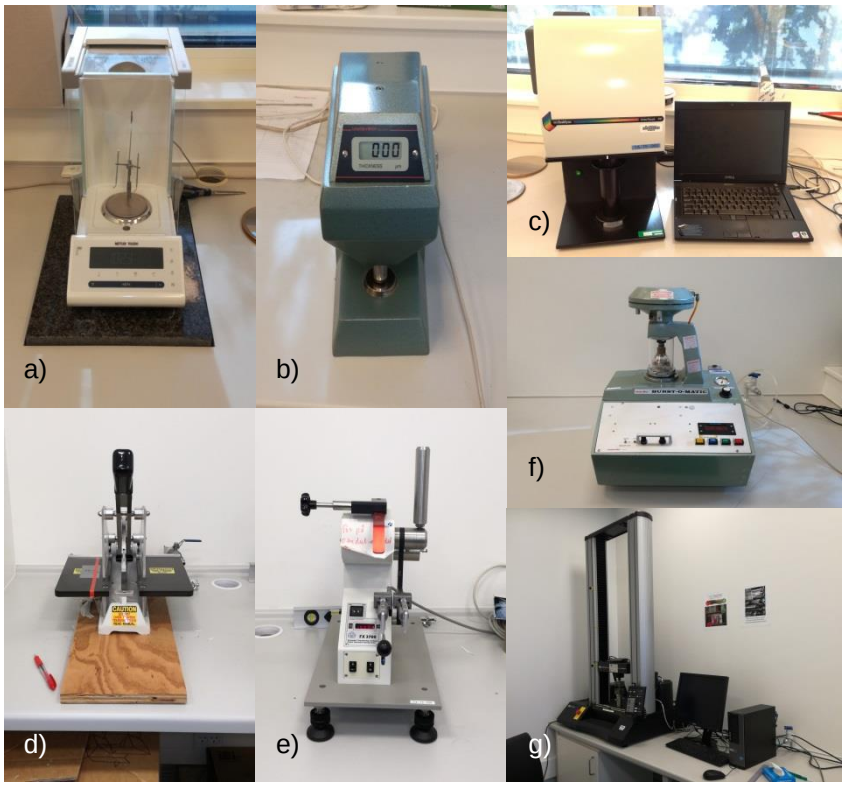

Figure 2.6 – Equipment used for the determination of basic, optical and physical properties: a) mass of individual handsheets, b) thickness of stacks, c) ISO brightness, d) cutting of handsheets for physical testing, e) tear strength, f) bursting strength, g) tensile strength.

All the measurements described herein serve the purpose of quantifying the physical strength of the handsheets. Table summarizes the indexes calculated from those measurements.

Table 2.2 – Paper properties, respective units and standards used to calculate them.

	Property	Units	Used standards
Basic properties	Basis weight	g/m^2	TAPPI Standard T414 om-98
	Apparent density	g/cm^3	TAPPI Standard T220
Optical properties	ISO brightness	%	ISO Standard 2470-2:2008
Physical properties	Burst strength index	kPa·m^2/g	TAPPI Standard T403 om-97
	Tear strength index	mN·m^2/g	TAPPI Standard T414 om-98
	Tensile strength index	N·m/g	TAPPI Standard T494 om-96

2.2.7 TOC

The TOC-V CPH (TOC-V) is developed by Shimadzu Corporation. It measures the amounts of total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC), the latter being the difference between TC and IC [21].

The filtrate sample, saved after the second draining as mentioned on page 22, was stored in a room at 5°C. It was first filtered to reduce fiber content in the mixture as much as possible, so as not to clog the TOC-V needle, with the recourse of a 20 mL syringe, used to push the suspension through a 0,45 mL filter and transferred to a vial for testing. The TOC-V datasheet was duly prepared for the assessment. The vial was placed in its allotted space in the carrousel. The software was set to start. The equipment had sensitivity to measure TOC in the range from 0 to 1000 mg/L in three segments: from 0 to 10 mg/L, from 10 to 100 mg/L, and from 100 to 1000 mg/L. A total of 27 samples were assessed in a single run of the TOC-V. These samples are related to the dosage curves, the refining curve for the bleached TMP, and the repeatability tests, experiments to be elaborated upon in the next section of this chapter.

Figure 2.7 – TOC-V CPH used for determining TOC.

2.3 Experiments

2.3.1 Basis weight optimization

As the heading state, the purpose was to get acquainted with handling the TMP and find out the BW to aim for and the revolution range in the PFI mill to test later on while ensuring the integrity of the handsheets. Methods used Table 2.3 showcases the values of those two parameters that were tested at this stage.

Table 2.3 – Parameters experimented upon and respective values tested during BW optimization.

Parameter	Values
Target BW (g/m^2)	60 (standard); 80; 100
Revolutions	0; 750; 1000; 1250; 1500; 2750; 5000

An additional challenge that had to be addressed was the removal of the handsheets from the mesh of the former without tearing them apart. A spatula had to be used to remove them every single time. For lower target basis weight and greater number of revolutions, this was still not enough. The solution was to add one more sheet of blotter paper in couching. Thus, 3 sheets would be placed upon the handsheet on the mesh before couching, instead of the standard two. Also, the stacks would have 7 handsheets with a target BW of 60 g/m2, but the number had to be reduced to 5 for both 80 and 100 g/m2 to have enough volume for pulp suspension tests. Both accepts and rejects were tested. A total of 83 handsheets were made at this stage.

2.3.2 Refining curves

The aim with doing refining curves was to study the effect refining would have on the physical properties mentioned on pages 27 and 27 and thus select the level of refining that maximizes these properties for implementation in the enzymatic treatment trials. Accepts, both bleached and unbleached, were tested. Target BW was 100 g/m^2. Number of revolutions tested were 750; 1000 and 1250; control samples for bleached and unbleached TMP weren't refined. A total of 40 handsheets were made for these trials, 4 stacks of 5 handsheets for both the bleached and unbleached TMP.

2.3.3 Dosage curves

The aim of these trials was to test enzymes manufactured by Novozymes at different concentrations and assess the impact they would have, one enzyme per trial. The dosage curves were made following the procedure described in the Methods sub-chapter. Bleached TMP was used for all of these trials. Target BW was 100 g/m^2. Number of revolutions in the PFI mill was 1000. Concentrations tested were 0,5; 1,0 and 2,5 kg/t odp. Milli-Q water was added to the control sample instead of enzyme solution (ES). Thus, 4 stacks of 5 handsheets were made in each trial. Table 2.4 and

Table 2.5 summarize the tested enzymes and the conditions of their respective dosage curve trials for TMP accept samples. Table 2.6 does the same for TMP reject samples.

Table 2.4 – Summary of the enzymes tested for dosage curves.

Enzyme	Subclass	Formulation
NS-51191	Xylanase	Liquid
NS-51184	Mannanase	Liquid
NS-51207	Xylanase	Liquid
NS-51180	Mannanase	Liquid
NS-51168	Xylanase	Liquid
NS-51137	Cellulase	Liquid
NS-51003	Laccase	Liquid

Table 2.5 – Enzymes tested for dosage curves on TMP accepts and the conditions under which they were tested.

Enzyme	Pre-incubation (min)	Incubation (min)	T (°C)	Buffer addition	Buffer pH	Buffer concentration (mM)
NS-51191	30	60	60	No	---	---
NS-51184	30	60	60	Yes	8	79,2
NS-51207	30	60	60	No	---	---
NS-51180	30	60	60	No	---	---
NS-51168	30	60	60	Yes	6	89,6
NS-51137	30	60	60	Yes	6	89,6
NS-51003	30	60	60	Yes	6	89,6

Table 2.6 – Enzymes tested for dosage curves on TMP rejects and the conditions under which they were tested.

Enzyme	Pre-incubation (min)	Incubation (min)	T (°C)	Buffer addition	Buffer pH	Buffer concentration (mM)
NS-51191	30	60	60	No	---	---
NS-51184	30	60	60	Yes	8	89,6

Three ES at the aforementioned concentrations were prepared. 600,00 mg of enzyme were weighed and transferred to a 100,0 mL volumetric flask. The flask was then filled to the mark with Milli-Q water. Thus, the ES at 2,5 kg/t odp was complete. 10,0 mL of this solution were pipetted to a 25,0 mL volumetric flask and then filled to the mark with Milli-Q water to prepare the ES at 1,0 kg/t odp. The ES at 0,5 kg/t odp was prepared in the same fashion, with 5,0 mL of the 2,5 kg/t odp ES, instead. These solutions were prepared while pre-incubation for the control sample was ongoing. The ES were then placed into iced water to prevent loss of enzyme activity while awaiting use. The samples would then be treated with 10,0 mL of one of the ES after the pre-incubation was done.

A total of 140 handsheets were made in these experiments. Schedule followed for handsheet preparation for each of these trials can be consulted in Appendix A, on page 66.

2.3.4 Repeatability trials

The aim of these trials was to confirm the results obtained in the dosage curves, as well as getting a sense of the effect a higher concentration would have on the physical properties in focus.

As such, the conditions for these trials are the same as of those for the dosage curves. The only change to point out is in the concentrations of the ES to treat the pulp samples: 0; 1,0; 2,5; 5,0 kg/t odp.

Table 2.7 – Enzymes tested for repeatability trials on TMP accepts and the conditions under which they were tested.

Enzyme	Pre-incubation (min)	Incubation (min)	T (°C)	Buffer addition	Buffer pH	Buffer concentration (mM)
NS-51180	30	60	60	No	---	---
NS-51003	30	60	60	Yes	8	89,6

Chapter 3 Results and Discussion

In this chapter, the main results regarding the experiments described in Chapter 2, are presented and interpreted in light of the body of work this project is based upon, as highlighted in Chapter 1.

3.1 Basis weight optimization and refining curves

It is well known MP are inherently weak when it comes to physical strength. This is because removal of lignin from the stock is not of interest for these pulps. Fortunately, it is possible to improve their physical resistance. The most widely used strategy for this purpose is refining, also known as beating. The forces the pulp is subjected to in this step peel away the outer layers of the fibers, where most of the lignin can be found, expose cellulose fibrils and collapse the fibers, thus increasing the surface area available for bonding. This happens at the expense of shortening of the fibers. How much stronger the paper gets as a result of refining depends on the extent to which each of these changes occur.

The low physical resistance of the TMP was indeed verified throughout the BW optimization experiments, with values of tensile, and tear strength indexes falling within the ranges between 7,73 and 10,42 Nm/g, and 4,34 and 5,73 mNm^2/g, for CSF values between 419,0 and 454,0 mL. Not one of these ranges coincide with the ones showcased in Figure 1.11, on page 13. Since the typical values of CSF are not greater than 300 mL, there is room for refining, which is expected to bring down the CSF figures while increasing the strength indexes. Thus, it is necessary to assess the effect changing the refining level has on the physical strength of the pulp.

Refining curves for bleached and unbleached TMP were generated. Levels of refining tested were 750, 1000 and 1250 revolutions. The control was subjected to no beating. Results are as follows.

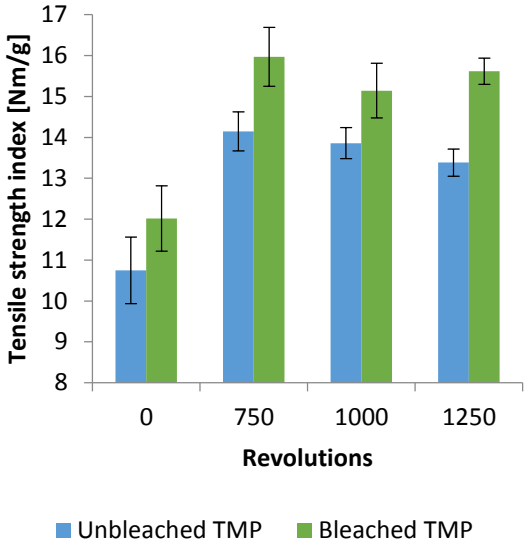

Figure 3.4 – Effect of refining level change on tensile strength index for unbleached and bleached TMP.

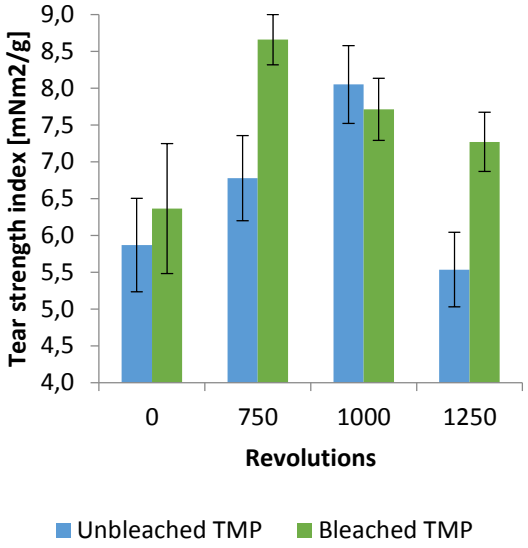

Figure 3.3 – Effect of refining level change on tear strength index for unbleached and bleached TMP.

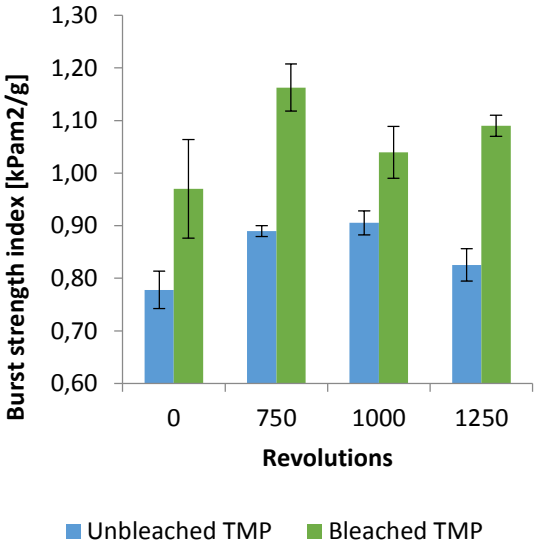

Figure 3.1 – Effect of refining level change on burst strength index for unbleached and bleached TMP.

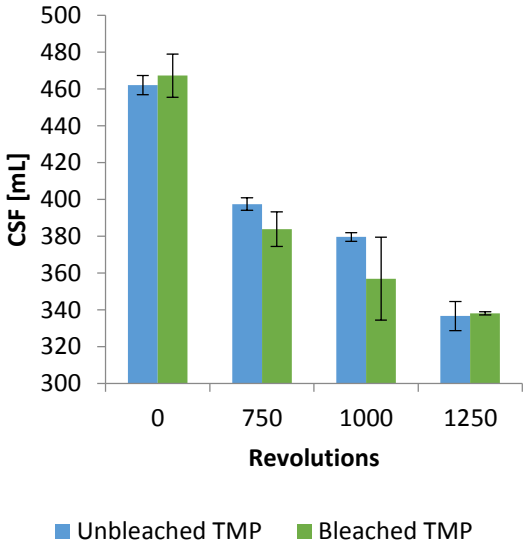

Figure 3.2 – Effect of refining level change on freeness for unbleached and bleached TMP.

Table 3.1 – Values of the basis weight, apparent density and ISO brightness for unbleached and bleached TMP samples refined at different levels.

	Unbleached TMP				Bleached TMP			
Revolutions	**0**	**750**	**1000**	**1250**	**0**	**750**	**1000**	**1250**
BW (g/m²)	99,88	94,55	95,33	92,24	92,93	94,20	88,52	93,15
App. density (g/cm³)	0,266	0,296	0,306	0,318	0,298	0,342	0,354	0,366
ISO brightness (%)	---	---	---	---	57,49	58,32	58,11	58,69

Table 3.2 – Fiber properties for unbleached and bleached TMP as the refining level increases.

	Unbleached TMP				Bleached TMP			
Revolutions	**0**	**750**	**1000**	**1250**	**0**	**750**	**1000**	**1250**
Crill	164,03	168,14	166,78	170,95	143,38	150,48	153,20	151,30
Length (mm)	1,94	1,74	1,71	1,59	1,74	1,86	1,65	1,63
Fines (%)	41,4	43,4	48,0	46,7	59,1	37,0	42,1	47,6
Width (µm)	33,6	33,5	33,8	35,0	33,6	35,0	34,2	35,1
Curl (%)	11,4	9,2	9,0	9,1	12,4	10,1	10,0	9,9
Kinks/mm	0,13	0,12	0,11	0,10	0,16	0,09	0,08	0,08

Looking at the plots in Figure 3.4, Figure 3.3 and Figure 3.1, it becomes apparent that refining improves the strength indexes for both unbleached and bleached TMP. In both cases, CSF decreases as the refining level increases (Figure 3.2) while the app. density increases (Table 3.1). This happens in spite of the values calculated of BW, which are lower than the target 100 g/m^2 and in some instances decreasing from one sample to the other, e.g. from the control unbleached sample to the one refined at 750 revolutions, or from the bleached sample refined at 750 revolutions to the one refined at 1000. This is expected, as cellulose fibrils get exposed after lignin is softened during refining, enabling better bonding between fibers.

In the case of unbleached TMP, both tensile and burst strength indexes, in Figure 3.4 and Figure 3.1, respectively, increase for 750 and 1000 to drop at 1250. The changes verified among the samples that were refined are not very significant, as opposed to what happens between the control and the 750 revolutions. To verify considerable deterioration of the handsheets from the physical standpoint, samples would have had to be subjected to more intense refining, a scenario that was not explored because the expected downward trend was already showing signs of happening. This worsening occurs in spite of the decrease of freeness/increase of app. density because parameters such as fiber length, fibrillation, as quantified by crill, and percentage of fines also influence the strength of paper, and refining can have significant effects on these factors. The results regarding those characteristics are presented in Table 3.2.

Crill, regarded as having a major influence in the outcome of the physical strength of the paper, increases from no refining to beating at 750 revolutions, decreases slightly for 1000 revolutions and increases to its highest value for 1250 revolutions. While greater fibrillation means bigger surface area, this didn't translate to better tensile and burst strength indexes because the length of the fibers decreased from 1,94 to 1,59 mm with the increase of the refining level. Increase of the surface area also happens with the increase of the amount of fines. For the unbleached samples, the greatest value for both indexes coincides with the highest percentage of fines, 48,0% for 1000 revolutions. This value, as well as those from the other three samples, are within the typical range of 40%-60%. In addition to this, fibers are also straightest for 1000 revolutions, with 9,0% of curl. There is also a steady decrease of the number of kinks per mm as the refining level increases. This contributes to the increase of surface area as well.

Another aspect that could have contributed for the increase of the surface area of the stock would have been fibers collapsing. This would have been reflected upon the fiber width; it would have decrease with refining. The opposite happened for the unbleached samples. This, in addition to what happened with the fiber length, indicates that the forces exerted in the PFI mill cut the fibers much more than they would collapse them.

Tear strength, unlike tensile and burst, doesn't depend so much on the interactions between fibers, but on the fibers themselves. The behavior pictured in this plot (Figure 3.3) is similar to that of tensile and burst, with the particularity of the changes between samples being more pronounced. Also, the values for the sample without beating and for 750 and 1000 are within the range of the typical values to be expected for TMP (from 6 to 8 mNm^2/g), while tensile underperforms significantly, when having Figure 1.10, on page 13, as a reference for comparison. This means that a set of conditions external to the pulp is affecting the inter-fiber interactions negatively.

Taking it all into consideration, the optimal refining level is between 750 and 1250 revolutions. Based on these results, 1000 revolutions were chosen to be applied in the subsequent dosage curve trials. The expectation was that the contribution of the enzymatic treatment would compound with this refining level and a more noticeable increase in strength would happen.

The refining curve with bleached TMP was generated after every dosage curve had been made. The intent was to confirm the expectation that bleaching with H_2O_2 would improve the physical strength indexes of the handsheets, particularly tensile and burst, which rely heavily on inter-fiber bonding. Bleaching in the case of MP targets lignin compounds known as chromophores, responsible for yellowing of paper made from these pulps over time and exposure to light, while minimizing mass losses as much as possible, so the benefit of high yield is not significantly affected. The modification of these compounds have the added benefit of exposing cellulose fibrils more, increasing the chances for interactions amongst themselves and subsequent bonding.

Regarding the results in Figure 3.3 and Figure 3.1, tensile and burst strength indexes for bleached TMP are indeed greater than those of the unbleached samples. Moreover, they are conclusively so, since the error bars from both unbleached and bleached TMP for the same refining level don't intercept one another, the exception being the tensile strength indexes for the control samples. This was verified for CSF values that were similar to their unbleached counterparts, except for 1000 revolutions, which was lower, even though it can be said that the real value might still be close to that of the unbleached sample refined at the same level, as showcased by the error bar of the former (Figure 3.2). Moreover, the app. density is greater for all the bleached samples, even though the mass loss was slightly bigger than in the unbleached refining curve, overall (Table 3.1). The increase on strength indexes happens despite fiber characteristics not being definitely better overall for the bleached samples (Table 3.2): for instance, in the case of the control sample, a big percentage of

fines, 59,1%, was counterbalanced with a shorter fiber length (1,74 mm) and less fibrillation (143,38). Fibrillation, overall, is worse in the case of the bleached samples. Fiber length and percentage of fines are also worse compared to those of the unbleached samples. In the case of tensile, bleaching also had the positive effect of raising the values to meet the lower end of the range that is typical for TMP for a greater value of CSF; not so much refining is needed to get somewhat acceptable results. Thus, bleaching has a significant effect on the physical strength of the handsheets, and it's not better because fiber characteristics are impacted negatively. This means that, on first basis, the bleaching step was effective and, consequently, its implementation a good decision.

Still, it is important to point out that some reserve is needed in how positive these results really are. First, there are several factors that influence the properties the paper ends up having, as clarified before. Adding bleaching to the procedure is adding one more factor to consider when critiquing the results. As such, uncertainty is bigger. Moreover, having added H_2O_2 to the pulp stock changes the conditions of that suspension, thus changing the way refining may affect the pulp. It may even shift the range where the optimal refining level may be found. This seems to have been the case when considering what happened at 1000 revolutions. For the three indexes in analysis, the bleached sample wasn't the one to present better values over the other refining levels tested. In fact, the best values for all of them were obtained at 750 revolutions. That being said, it is not possible to say the curve was shifted to the left in all cases, since both tensile and burst at 1250 revolutions had better values than at 1000 revolutions, which is not what would have happened, if there had been an actual shift of the curve.

Table 3.3 – TOC, TC and IC for bleached TMP samples with refining level changes.

Enzyme	**Bleached TMP**			
Revolutions	0	750	1000	1250
TOC (mg/L)	32,23	32,12	29,87	32,04
TC (mg/L)	51,71	52,53	52,65	52,85
IC (mg/L)	19,48	20,42	22,78	20,81

TOC was also measured for bleached TMP. Since there was no enzymatic treatment, the expectation was that the value remained approximately the same for all samples. That is effectively the case.

3.2 Dosage curves

Once the refining curve was generated and the refining level chosen, the effect of varying ES concentration was studied for several enzymes. These enzymes were xylanases, mannanases, a cellulose and a lacasse, as listed in Table 2.4, on page 30. Comparisons between enzymes of the same subclass are made in the case of xylanases and mannanases. The dosage curves for xylanases, mannanases, the cellulase and the laccase are looked into separately. This subchapter focuses solely on

the first dosage curves made for each of the enzymes tested. Repeatability trials are addressed later in this chapter.

For these dosage curves, the tested ES concentrations were 0,50; 1,00 and 2,50 kg/t odp. The implemented refining level was 1000 revolutions. All the tests were performed on TMP bleached with H_2O_2. For controls, ES concentration = 0 kg/t odp.

3.2.1 Xylanases

The tested xylanases in these trials were NS-51191 (60 min, 60°C), NS-51207 (60 min, 60°C) and NS-51168 (60 min, 60°C, buffer pH = 6). Results are as follows.

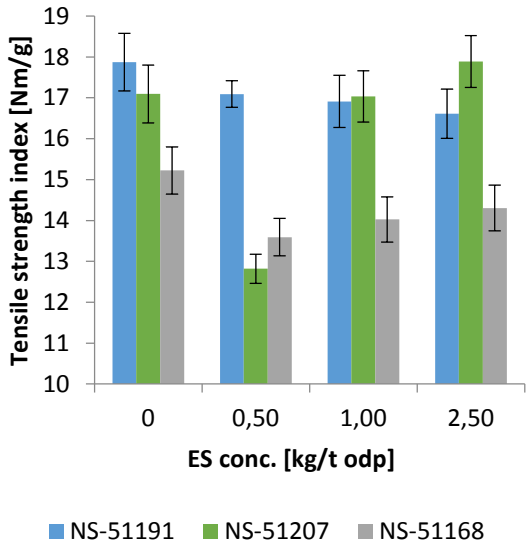

Figure 3.8 – Effect of ES concentration change on tensile strength index for the tested xylanases on bleached TMP.

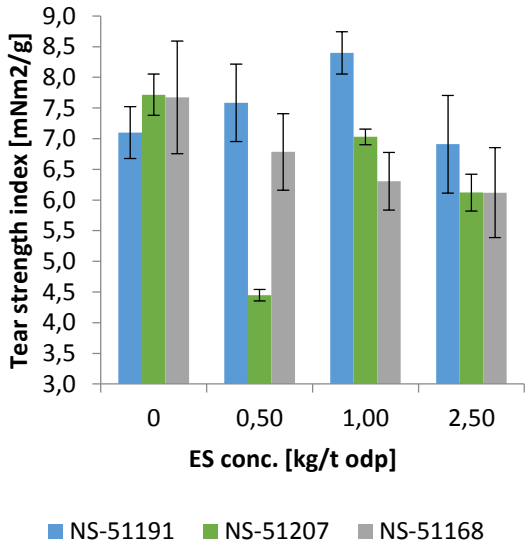

Figure 3.7 – Effect of ES concentration change on tear strength index for the tested xylanases on bleached TMP.

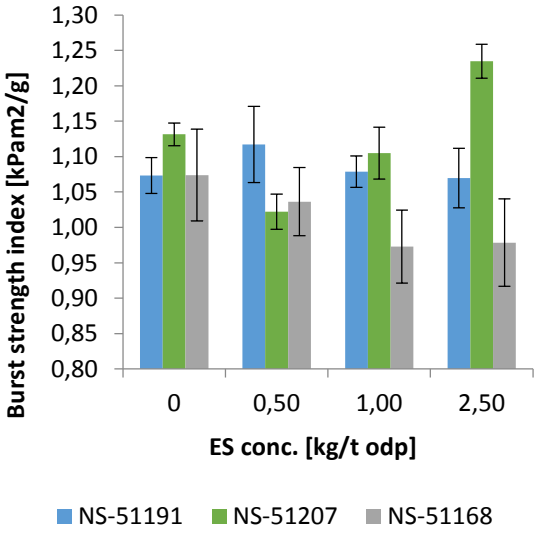

Figure 3.6 – Effect of ES concentration change on burst strength index for the tested xylanases on bleached TMP.

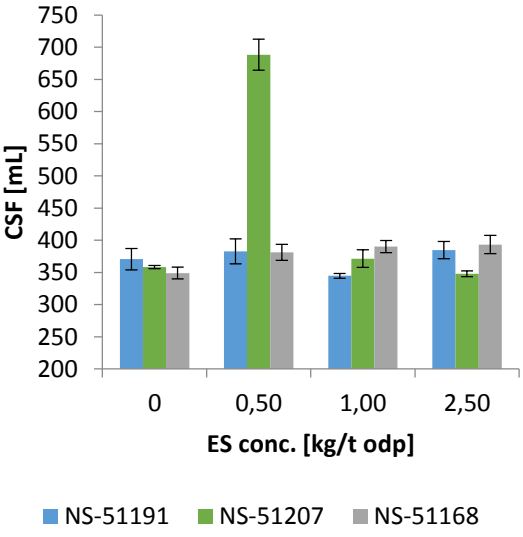

Figure 3.5 – Effect of ES concentration change on freeness for the tested xylanases on bleached TMP.

Table 3.4 – Values of the strength indexes and freeness for the control samples and gains for each tested ES concentration for the tested xylanases on bleached TMP.

Enzyme	**NS-51191**				**NS-51207**				**NS-51168**			
ES conc. (kg/t odp)	**0**	**0,50**	**1,00**	**2,50**	**0**	**0,50**	**1,00**	**2,50**	**0**	**0,50**	**1,00**	**2,50**
Tensile (Nm/g)	17,87	-4,31%	-5,37%	-7,02%	17,10	-25,01%	-0,38%	4,64%	15,22	-10,71%	-7,87%	-6,03%
Tear (mNm^2/g)	7,10	6,84%	18,31%	-2,67%	7,72	-40,25%	-6,19%	-17,73%	7,67	-11,57%	-17,79%	-20,24%
Burst ($kPam^2/g$)	1,07	4,10%	0,51%	-0,34%	1,13	-9,65%	-2,33%	9,13%	1,07	-3,50%	-9,40%	-8,89%
CSF (mL)	370,6	3,27%	-6,98%	3,82%	358,5	92,00%	3,65%	-2,90%	349,1	9,19%	11,79%	12,65%

Table 3.5 – Values of the basis weight, apparent density and ISO brightness for unbleached and bleached TMP samples refined at different levels.

Enzyme	**NS-51191**				**NS-51207**				**NS-51168**			
ES conc. (kg/t odp)	**0**	**0,50**	**1,00**	**2,50**	**0**	**0,50**	**1,00**	**2,50**	**0**	**0,50**	**1,00**	**2,50**
BW (g/m^2)	90,88	87,52	98,98	85,44	90,19	49,46	84,07	85,11	94,07	92,78	88,15	85,73
App. density (g/cm^3)	0,345	0,358	0,357	0,355	0,347	0,394	0,357	0,392	0,357	0,362	0,360	0,362
ISO brightness (%)	58,72	58,57	58,23	59,65	57,58	54,55	57,51	57,11	56,70	57,77	58,41	58,14

Table 3.6 – Fiber characteristics for all the tested ES concentrations and xylanases in bleached TMP.

Enzyme	**NS-51191**				**NS-51207**				**NS-51168**			
ES conc. (kg/t odp)	**0**	**0,50**	**1,00**	**2,50**	**0**	**0,50**	**1,00**	**2,50**	**0**	**0,50**	**1,00**	**2,50**
Crill	153,88	156,39	155,63	152,99	153,08	148,27	153,32	154,90	150,87	149,69	149,60	151,05
Length (mm)	1,75	1,68	1,72	1,78	1,81	1,58	1,67	1,68	1,75	1,72	1,75	1,64
Fines (%)	40,7	44,6	45	44,4	38,3	43,2	52,7	47,1	41,2	38,9	46	40,2

Width (µm)	35,1	34,0	34,0	34,8	34,8	34,9	34,8	34,8	33,3	34,7	34,1	34,4
Curl (%)	9,6	9,4	9,4	9,6	9,0	9,2	9,3	10,1	9,3	9,0	9,6	9,7
Kinks /mm	0,11	0,11	0,10	0,12	0,10	0,12	0,11	0,10	0,12	0,11	0,11	0,11

Table 3.7 – TOC, TC and IC for all the tested ES concentrations and xylanases in bleached TMP.

En-zyme	**NS-51191**				**NS-51207**				**NS-51168**			
ES conc. (kg/t odp)	0	0,50	1,00	2,50	0	0,50	1,00	2,50	0	0,50	1,00	2,50
TOC (mg/L)	16,20	36,42	70,12	92,79	25,74	15,74	32,99	49,12	600,8	606,3	588,3	639,3
TC (mg/L)	38,33	56,20	89,36	113,10	45,53	34,80	50,77	70,85	601,2	606,7	588,7	639,7
IC (mg/L)	22,13	19,77	19,24	20,30	19,78	19,06	17,79	21,73	0,43	0,39	0,42	0,40

For NS-51191, burst strength index starts at 1,07 kPam2/g, reaches the maximum at 0,50 kg/t odp, with a 4,10% increase, then decreases for both 1,00 and 2,50 kg/t odp to values similar to that of the control (Figure 3.6, Table 3.4). the same behavior is seen on Table 3.7 for crill. The percentage of fines also increases from 40,7% to 44,6% for the same concentration and remains steady for the other two samples. Since these two parameters are the ones that influence inter-fiber bonding the most and burst and tensile depend a lot on this, it would be reasonable to expect tensile strength indexes to behave in a similar manner. That's not the case, though. Tensile strength index starts at 17,78 Nm/g and decreases slightly and steadily, up to 7,02% at 2,50 kg/t odp, as the ES concentration increases (Table 3.5, Table 3.4). This may occur because bonding is not uniform throughout the handsheets and different sections are used for assessing these parameters. Other parameters that can also contribute to inter-fiber bonding, i.e. fiber width, curl and kinks/mm, don't experience significant changes.

The tear strength indexes verify growth until 1,00 kg/t odp, where it is 18,31%, Then drop below the control at 2,50 kg/t odp (Figure 3.7, Table 3.4). This doesn't coincide with the results on fiber length, to which tear is strongly related and decreases at 0,50 kg/t odp then increases. The displayed behavior does resemble more closely what happens with the BW, which is the highest at 1,00 kg/t odp, while also taking into account the app. density is very similar among the three enzyme-treated samples (Table 3.5).

CSF values don't show a clear trend, since samples at 0,50 and 2,50 kg/t odp experience growths of 3,27% and 3,82%, respectively, unlike the sample treated with ES at 1,00 kg/t odp, which

decreases 6,98% (Figure 3.5, Table 3.4). This mirrors the results of the BW, which are higher for the sample treated with ES at 1,00 kg/t odp. Also, these results show mass was lost while preparing the handsheets. These losses most likely happened every time it was necessary to transfer the pulp stock between beakers in the different steps of bleaching, handsheet preparation and pulp suspension testing.

While the interdependence between all these factors is known and has been addressed, it is necessary to know whether NS-51191 plays any part in these data. Looking at TOC on Table 3.7, there is a clear increase as the ES concentration increases, as corroborated by the TC data, which also increases, while IC remains constant. Since this parameter is measured on filtrates before refining occurs, it is safe to say NS-51191 had an effect on the pulp tested. It is difficult to say to which extent it did, though, because the data put forth consists of average values and variability is bound to happen, despite the conditions under which the tests were conducted are the same. This is addressed later.

Similar reasoning can be applied to interpret the results of the other two xylanases. CSF is strongly linked to BW, but also to app. density. While usually lower BW means lower CSF, if density increases significantly, it compensates for a lower BW, as in the sample treated with 1,00 kg/t odp of NS-207 solution. When crill is constant, changes in tensile and/or burst can be attributed to differences in the percentage of fines, whose presence is strongly related to mechanical action, and not enzyme treatment, since they can only act on the surface of the fibers due to the specificity of the pulp at study, which keeps all the main components of wood. Fiber length also needs to be taken into consideration, as well as fiber width, curl and kinks.

Still, it is important to highlight outliers, as is the case with the sample treated with NS-51207. The value of CSF, which nearly doubles tat of the control, as well as the very low tensile and tear strength indexes have to do with the BW calculated for that sample, 49,46 g/m^2, which is half of the target 100 g/m^2. With such loss of mass, fibers didn't interact properly with one another, thus producing very weak handsheets that would let the water drain very easily. The amount of mass that was lost is due to human error in the bleaching step, either when weighing the pulp to be treated, or during washing.

The results on TOC for NS-51207 and NS-51168 (Table 3.4) differ greatly in terms of magnitude. They both have one thing in common, though: the enzyme did not respond in a remarkable way, since the values did not change significantly as the concentration increased. In the specific case of NS-51168, TOC is around 600 mg/L for all samples. This is very different from NS-51191 and NS-51207, none of which is greater than 100 mg/L. This discrepancy cannot be attributed to anything related to the enzyme, though. For that to be possible, the values of TOC for the control would have to be much different, which is isn't. This increase in TOC may have happened during storage at 5°C, while it awaited testing for several days. A smaller waiting period between bleaching and handsheet

preparation would solve this issue. The high content in organic carbon might have also hindered the action of NS-51168. In the case of NS-51207, the lack of responsiveness may be due to a pH that might have been too alkaline, due to NaOH that might have remained with the pulp after washing. Specifically, pH was 8,10, when the dosage curve was made without buffer, expecting the pH to be in the range of 7,30 – 7,70, as measured throughout the BW optimization trials with unbleached TMP. Working with a buffer at pH = 7 could discard this as an issue.

In conclusion, care needs to be taken to ensure the intended conditions for incubation and discard that as a potential problem. In the case of NS-51191, while there was some responsiveness, the obtained values didn't improve to an extent that it would be productive to follow-up on them. Also, the hopes of seeing trends common to these xylanases as a result of belonging to the same subclass were not met.

3.2.2 Mannanases

The tested mannanases in these trials were NS-51184 (60 min, 60°, buffer pH = 6) and NS-51180 (60 min, 60°C). Results are as follows.

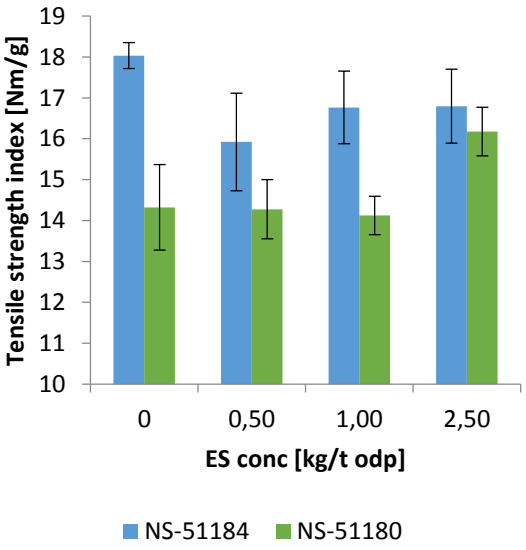

Figure 3.10 – Effect of ES concentration change on tensile strength index for the tested mannanases on bleached TMP.

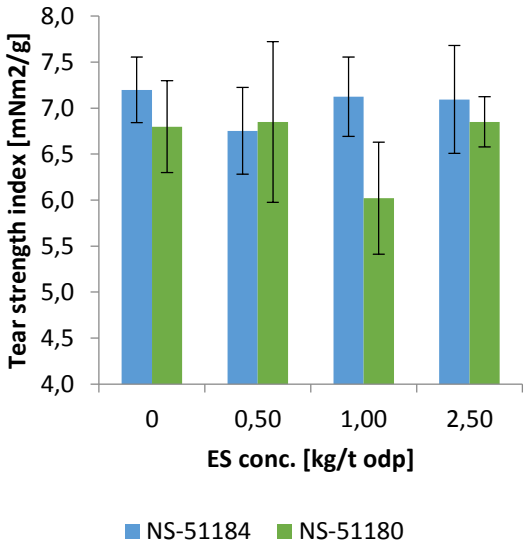

Figure 3.9 – Effect of ES concentration change on tear strength index for the tested mannanases on bleached TMP.

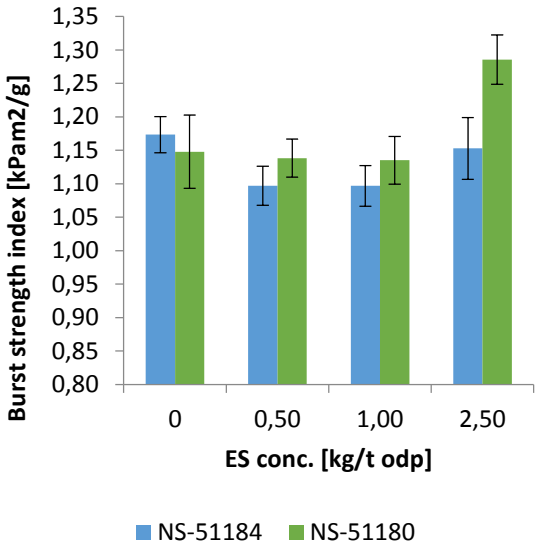

Figure 3.12 – Effect of ES concentration change on burst strength index for the tested mannanases on bleached TMP.

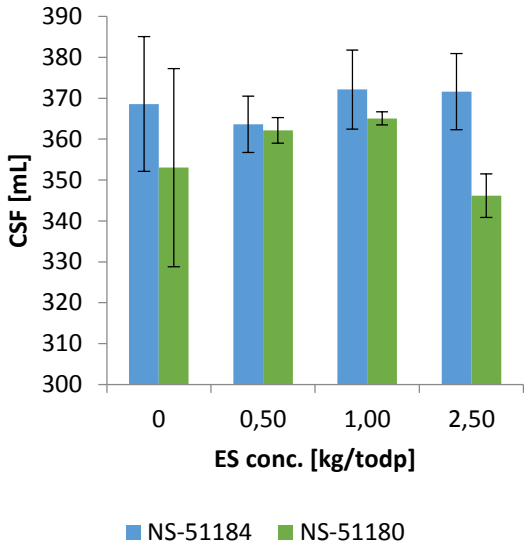

Figure 3.11 – Effect of ES concentration change on freeness for the tested mannanases on bleached TMP.

Table 3.8 – Values of the strength indexes and freeness for the control samples and gains for each tested ES concentration for the tested mannanases on bleached TMP.

Enzyme	NS-51184				NS-51180			
ES conc. (kg/t odp)	0	0,50	1,00	2,50	0	0,50	1,00	2,50
Tensile (Nm/g)	18,03	-11,71%	-7,03%	-6,86%	14,32	-0,34%	-1,41%	12,92%
Tear (mNm^2/g)	7,20	-6,17%	-1,03%	-1,43%	6,80	0,76%	-11,41%	0,77%
Burst (kPam^2/g)	1,17	-6,51%	-6,52%	-1,75%	1,15	-0,84%	-1,12%	11,99%
CSF (mL)	368,6	-1,34%	0,96%	0,82%	353,0	2,59%	3,41%	-1,94%

Table 3.9 – Values of the basis weight, apparent density and ISO brightness for each tested ES concentration for the tested mannanases on bleached TMP.

Enzyme	NS-51184				NS-51180			
ES conc. (kg/t odp)	0	0,50	1,00	2,50	0	0,50	1,00	2,50
BW (g/m^2)	89,24	87,42	84,49	83,53	88,81	84,53	82,28	86,14
App. density (g/cm^3)	0,353	0,356	0,352	0,362	0,351	0,354	0,364	0,360
ISO brightness (%)	58,67	59,15	56,99	58,59	56,70	57,77	58,41	58,14

Table 3.10 – Fiber characteristics for all the tested ES concentrations and mannanases in bleached TMP.

Enzyme	NS-51184				NS-51180			
ES conc. (kg/t odp)	0	0,50	1,00	2,50	0	0,50	1,00	2,50
Crill	153,88	156,39	155,63	152,99	154,44	157,30	154,06	156,43
Length (mm)	1,80	1,69	1,69	---	1,77	1,69	1,72	1,71
Fines (%)	39,7	41,7	42,4	---	35,3	37,9	50,1	49,1
Width (µm)	33,1	34,9	34,6	---	33,3	33,7	34	33,8
Curl (%)	9,2	8,6	8,7	---	9,2	9,1	9,2	9,9
Kinks/mm	0,11	0,09	0,09	---	0,11	0,10	0,11	0,11

Table 3.11 – TOC, TC and IC for all the tested ES concentrations and mannanases in bleached TMP.

Enzyme	**NS-51184**				**NS-51180**			
ES conc. (kg/t odp)	0	0,50	1,00	2,50	0	0,50	1,00	2,50
TOC (mg/L)	382,7	439,5	399,4	366,1	24,54	25,39	29,95	40,84
TC (mg/L)	386,3	442,9	402,2	369,2	46,54	46,13	49,07	61,21
IC (mg/L)	3,59	3,44	2,83	3,07	22,01	20,74	19,12	20,36

The analysis of Figure 3.10, Figure 3.9 and Figure 3.12 showcase similar behaviors of NS-51184 and NS-51180 to those of NS-51191 and NS-51207. For NS-51184, there is a decrease in the tensile strength index from the control to 0,50 kg/t odp. Simultaneously, the greatest value for TOC, 439,5 mg/L, was verified for this sample. For higher concentrations, there seems to be a slight increase, but that can be accounted by the variability displayed. A similar pattern is verified for burst strength, although less pronounced. In the case of NS-51180, an increase in both tensile and burst strength indexes occurs for the 2,50 kg/t odp treated sample, remaining essentially unchanged for lower concentrations. This is supported by the increase in TOC for this concentration, in comparison to that of the control and the other concentrations, which remain constant, essentially. The one noticeable difference is that tensile strength index for the samples treated with NS-51184 is greater for every concentration than that of the samples treated with NS-51180, which doesn't happen for the burst strength indexes.

Variability continues to be more expressive for tear strength index, making it difficult to assert conclusively about the observed changes. Even disregarding this factor, it is clear that there were not any significant changes in the case of NS-51180. For NS-51180, there was a decrease for the 1,00 kg/t odp-treated sample. The rest remained unchanged in comparison to the control.

When it comes to freeness, app. density and BW, displayed in Figure 3.11 and Table 3.9, respectively, results are more consistent with one another than in the xylanase trials. This means that the peak observed might be either to the enzyme or to other characteristics of the pulp, such as fiber length or percentage of fines.

Since the increase verified for the sample treated with NS-51180 at 2,50 kg/t odp was over 10% for both tensile and burst, the results showcased here are to be confirmed in a new trial, unlike NS-51184, which had a negative effect on these indexes, making it not worthy to explore any further.

3.2.3 Cellulase

NS-51137 (60 min, 60°C, pH = 6) was the cellulase tested. Results are as follows.

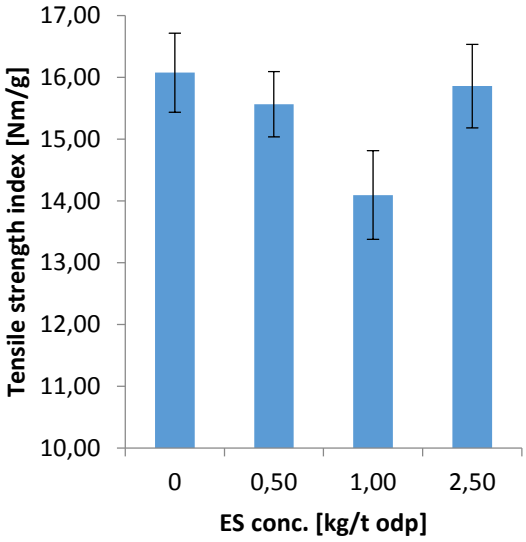

Figure 3.16 – Effect of ES concentration change on tensile strength index for the tested cellulase on bleached TMP.

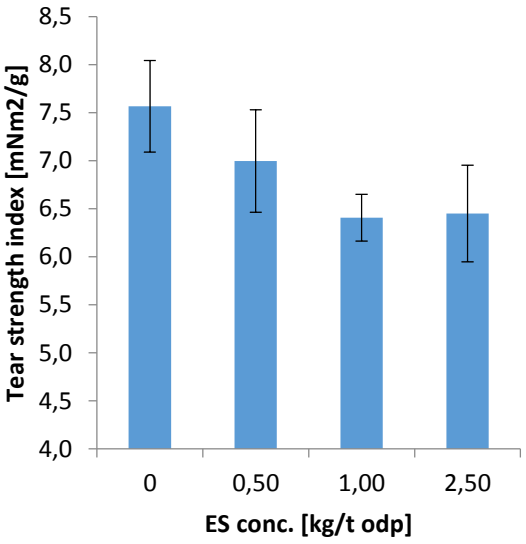

Figure 3.15 – Effect of ES concentration change on tear strength index for the tested cellulase on bleached TMP.

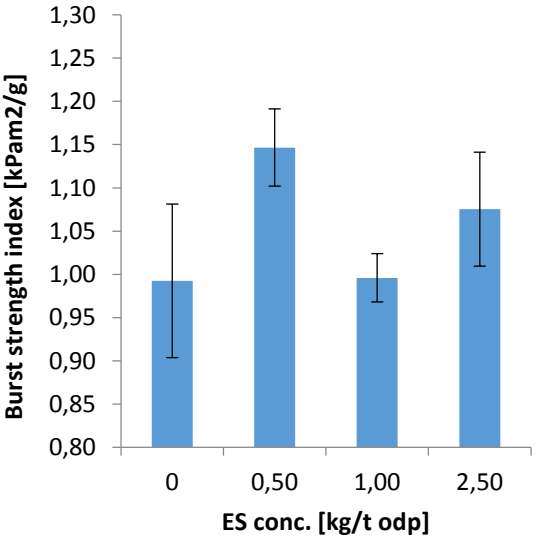

Figure 3.13 – Effect of ES concentration change on burst strength index for the tested cellulase on bleached TMP.

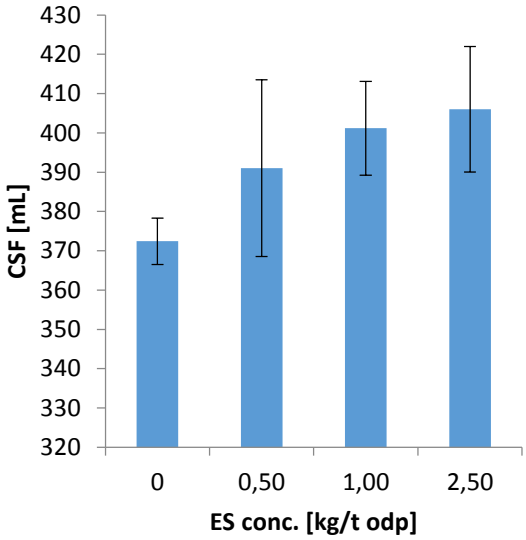

Figure 3.14 – Effect of ES concentration change on freeness for the tested cellulase on bleached TMP.

Table 3.12 – Values of the strength indexes and freeness for the control samples and gains for each tested ES concentration for the tested cellulase on bleached TMP.

Enzyme	**NS-51137**			
ES conc. (kg/t odp)	**0**	**0,50**	**1,00**	**2,50**
Tensile (Nm/g)	16,08	-3,17%	-12,32%	-1,35%
Tear (mNm^2/g)	7,57	-7,53%	-15,31%	-14,75%
Burst (kPam^2/g)	0,99	15,53%	0,34%	8,35%
CSF (mL)	372,4	4,99%	7,72%	9,02%

Table 3.13 – Values of the basis weight, apparent density and ISO brightness for each tested ES concentration for the tested cellulase on bleached TMP.

Enzyme	**NS-51137**			
ES conc. (kg/t odp)	**0**	**0,50**	**1,00**	**2,50**
BW(g/m^2)	93,82	91,64	88,77	89,21
App. density (g/cm^3)	0,352	0,358	0,341	0,383
ISO brightness (%)	57,11	56,55	56,21	58,17

Table 3.14 – Fiber characteristics for all the tested ES concentrations and cellulase in bleached TMP.

Enzyme	**NS-51137**			
ES conc. (kg/t odp)	**0**	**0,50**	**1,00**	**2,50**
Crill	145,79	148,91	149,67	150,94
Length (mm)	1,97	1,74	1,67	1,70
Fines (%)	56,4	48,8	51,8	43,1
Width (µm)	35,4	35,6	33,4	34,8
Curl (%)	9,4	9,5	9,3	9,6
Kinks/mm	0,11	0,10	0,10	0,10

Table 3.15 – TOC, TC and IC for all the tested ES concentrations and cellulose in bleached TMP.

Enzyme	**NS-51137**		
ES conc. (kg/t odp)	0,50	1,00	2,50
TOC (mg/L)	471,7	515,3	497,2
TC (mg/L)	472,1	515,7	497,5
IC (mg/L)	0,43	0,47	0,34

Monocomponent endoglucanases are known to shorten fibers. This should translate into lower tear strength indexes, as well as greater CSF. This is what effectively happens in Figure 3.15 and Figure 3.14. In Table 3.14, the expected fiber shortening can be seen as the ES concentration increases. This trend can be also seen on how the percentage of fines changes with the increase of concentration. On the other hand, fibrillation increases with the increase of the ES concentration, although not as significantly. These factors reflect on the way tensile varies with concentration, from the control sample to the sample at 1,00 kg/t odp, the it increases back to the level of the control. Burst strength indexes don't show the same behavior as tensile strength indexes. This is probably because of an uneven inter-fiber bonding throughout the handsheets, as explained in the Xylanases section.

All in all, the results obtained for NS-51137 correspond to expectations. Since the effects are negative, no further experimentation is to be performed with this enzyme.

3.2.4 Laccase

NS-51003 (60 min, 60°C, buffer pH = 6) was the laccase tested. Results are as follows.

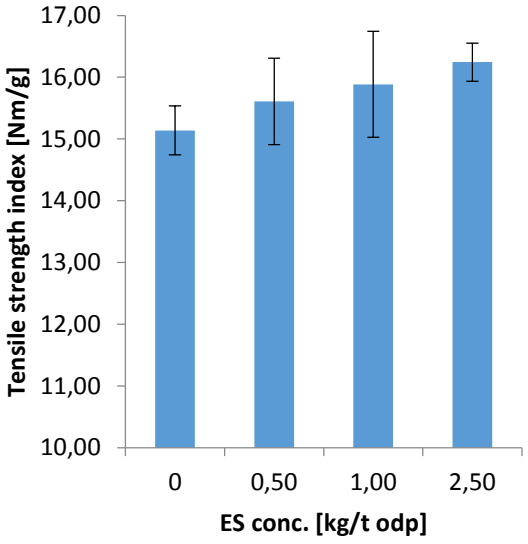

Figure 3.18 – Effect of ES concentration change on tensile strength index for the tested laccase on bleached TMP.

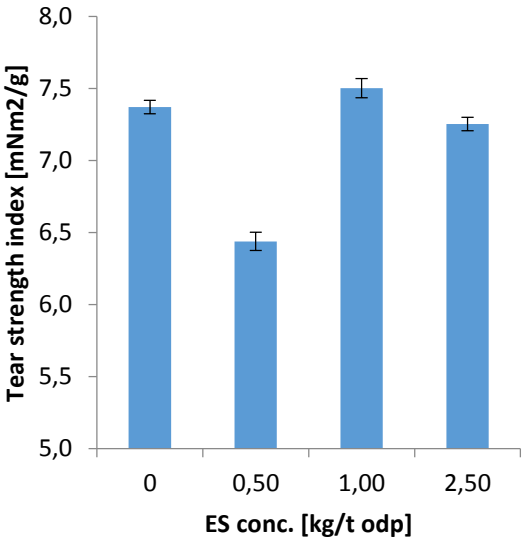

Figure 3.17 – Effect of ES concentration change on tear strength index for the tested laccase on bleached TMP.

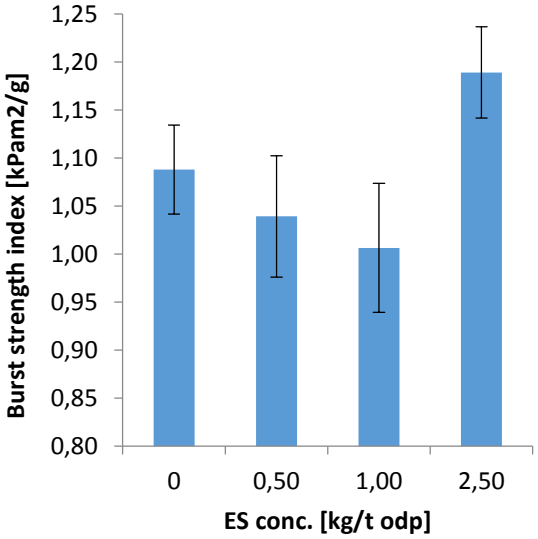

Figure 3.20 – Effect of ES concentration change on burst strength index for the tested laccase on bleached TMP.

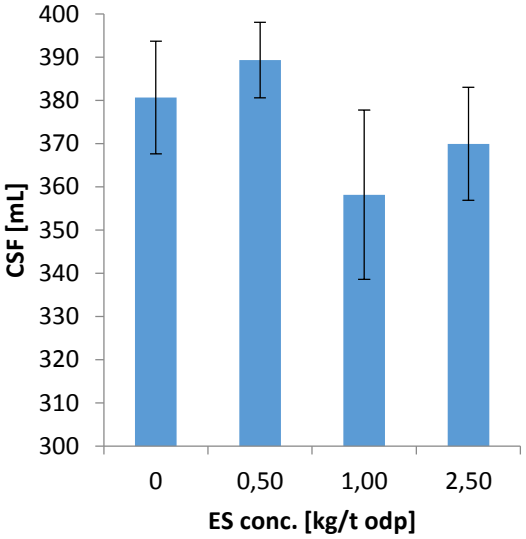

Figure 3.19 – Effect of ES concentration change on freeness for the tested laccase on bleached TMP.

Table 3.16 – Values of the strength indexes and freeness for the control samples and gains for each tested ES concentration for the tested laccase on bleached TMP.

Enzyme	**NS-51003**			
ES conc. (kg/t odp)	**0**	**0,50**	**1,00**	**2,50**
Tensile (Nm/g)	15,14	3,11%	4,93%	7,31%
Tear (mNm^2/g)	7,37	-4,49%	-7,50%	9,28%
Burst ($kPam^2/g$)	1,09	-4,49%	-7,50%	9,28%
CSF (mL)	380,6	-2,78%	3,22%	1,63%

Table 3.17 – Values of the basis weight, apparent density and ISO brightness for each tested ES concentration for the tested cellulase on bleached TMP.

Enzyme	**NS-51003**			
ES conc. (kg/t odp)	**0**	**0,50**	**1,00**	**2,50**
BW (g/m^2)	93,82	91,64	88,77	89,21
App. density (g/cm^3)	0,352	0,358	0,341	0,383
ISO brightness (%)	58,08	57,35	56,20	56,49

Table 3.18 – Fiber characteristics for all the tested ES concentrations and laccase in bleached TMP.

Enzyme	**NS-51003**			
ES conc. (kg/t odp)	**0**	**0,50**	**1,00**	**2,50**
Crill	150,52	151,39	150,54	156,40
Length (mm)	1,92	1,72	1,76	1,61
Fines (%)	45,4	53,8	56,1	42
Width (µm)	34,8	34,8	35,5	34,5
Curl (%)	9,3	9,4	9,6	9,4
Kinks/mm	0,12	0,11	0,08	0,09

Table 3.19 – TOC, TC and IC for all the tested ES concentrations and laccase in bleached TMP.

Enzyme	**NS-51003**			
ES conc. (kg/t odp)	0	0,50	1,00	2,50
TOC (mg/L)	417,9	527,3	49,47	561,3
TC (mg/L)	418,4	527,7	70,43	561,7
IC (mg/L)	0,47	0,46	20,96	0,46

Results of tensile, tear and burst strength indexes are displayed in Figure 3.18, Figure 3.17 and Figure 3.20, respectively. In the case of tensile, a steady, if gradual, increase is observed. This is congruent with the action laccases have been reported to have, attacking colloidal substances, which usually make it more difficult for fiber to bond amongst themselves. A slight decrease in ISO brightness cam also be conferred in Table 3.17. This was also expected.

Burst strength indexes do not behave like tensile; they decrease up to 1,00 kg/t odp and then increase at 2,50 kg/t odp to an extent that can be considered significant in comparison to the control. In the case of tear, there is not a steady trend that can be discerned from the plot. The sample treated with 2,50 kg/t odp ES does not register significant changes in comparison to the control. Samples treated with 0,50 and 1,00 kg/t odp ES register a decrease and an increase, respectively. These results mirror the behavior for freeness and app. density, Table 3.17. The loss of mass, Table 3.18, is on par with the results previously discussed.

Due to the results in tensile, it was decided NS-51003 would be tested to confirm the results here presented.

3.3 Repeatability trials

The results generated in the dosage curves weren't very promising. Among all the enzymes tested, NS-51180 and NS-51003 were considered for further testing. The first step in this direction is to confirm the results obtained in the first dosage curves. As such, conditions were maintained. The exception is in the concentrations of the ES to be added. Since increases were verified for higher concentrations. It was decided 5,00 kg/t odp would replace 0,50 kg/t odp in order to assess if the increase would continue for higher concentrations and to which extent.

3.3.1 NS-51180

Results regarding the comparison between the first dosage curve and the repeatability trial for NS-51180 are as follows.

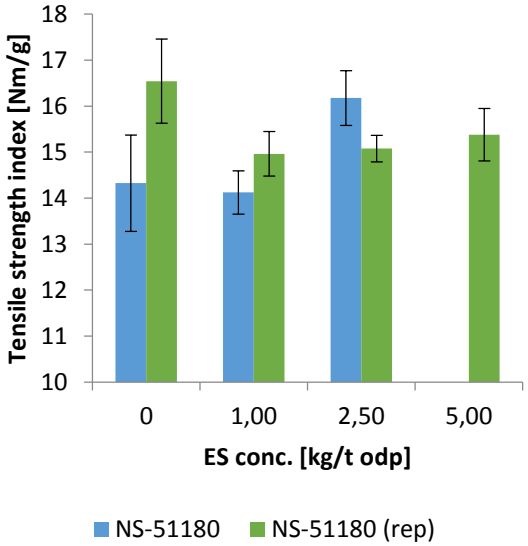

Figure 3.22 – Effect of ES concentration change on tensile strength index for NS-51180 on bleached TMP.

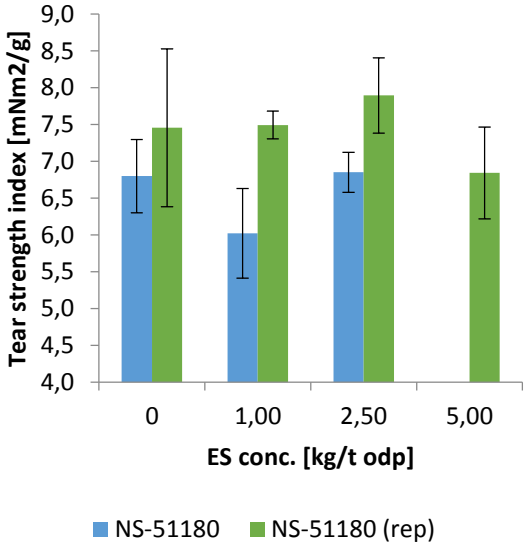

Figure 3.21 – Effect of ES concentration change on tear strength index for NS-51180 on bleached TMP.

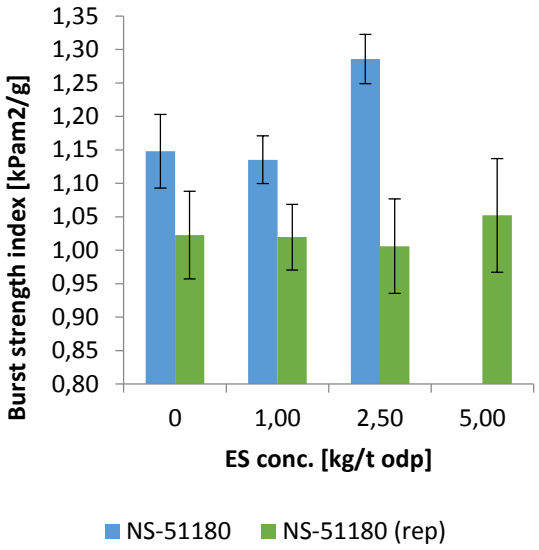

Figure 3.24 – Effect of ES concentration change on burst strength index for NS-51180 on bleached TMP.

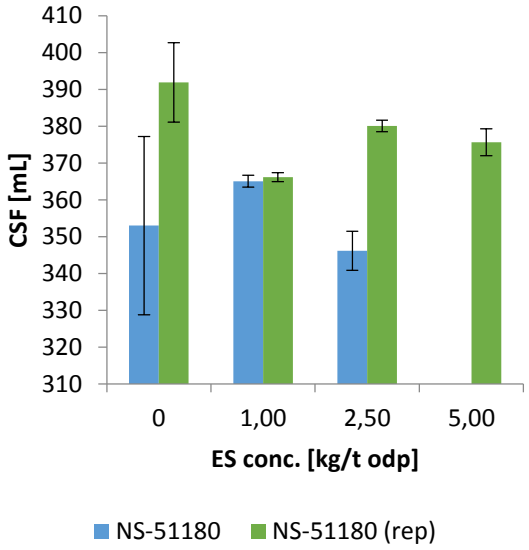

Figure 3.23 – Effect of ES concentration change on freeness for NS-51180 on bleached TMP.

Table 3.20 – Values of the strength indexes and freeness for the control samples and gains for each tested ES concentration for NS-51180 on bleached TMP.

Enzyme	NS-51180				NS-51180 (rep)			
ES conc. (kg/t odp)	0	0,50	1,00	2,50	0	1,00	2,50	5,00
Tensile (Nm/g)	14,32	-0,34%	-1,41%	12,92%	16,54	-9,55%	-8,87%	-7,04%
Tear (mNm²/g)	6,80	0,76%	-11,41%	0,77%	7,45	0,52%	5,90%	-8,23%
Burst (kPam²/g)	1,15	-0,84%	-1,12%	11,99%	1,02	-0,84%	-1,12%	11,99%
CSF (mL)	353,0	2,59%	3,41%	-1,94%	392,0	-6,58%	-3,04%	-4,16%

Table 3.21 – Values of the basis weight, apparent density and ISO brightness for each tested ES concentration for NS-51180 on bleached TMP.

Enzyme	NS-51180				NS-51180 (rep)			
ES conc. (kg/t odp)	0	0,50	1,00	2,50	0	1,00	2,50	5,00
BW (g/m²)	88,81	84,53	82,28	86,14	93,89	90,51	91,68	92,37
App. density (g/cm³)	0,351	0,354	0,364	0,360	0,345	0,346	0,342	0,356
ISO brightness (%)	56,70	57,77	58,41	58,14	57,95	58,01	58,29	57,91

Table 3.22 – Fiber characteristics for each tested ES concentration for NS-51180 on bleached TMP.

Enzyme	NS-51180				NS-51180 (rep)			
ES conc. (kg/t odp)	0	0,50	1,00	2,50	0	1,00	2,50	5,00
Crill	154,44	157,30	154,06	156,43	152,05	155,04	155,17	154,92
Length (mm)	1,77	1,69	1,72	1,71	1,80	1,80	1,78	1,79
Fines (%)	35,3	37,9	50,1	49,1	35,4	41,3	52,2	47,5
Width (µm)	33,3	33,7	34	33,8	34,0	35,7	32,7	33,6
Curl (%)	9,2	9,1	9,2	9,9	9,7	9,5	9,8	9,5
Kinks/mm	0,11	0,10	0,11	0,11	0,11	0,12	0,08	0,10

Table 3.23 – TOC, TC and IC for each tested ES concentration for NS-51180 on bleached TMP.

Enzyme	NS-51180				NS-51180 (rep)			
ES conc. (kg/t odp)	0	0,50	1,00	2,50	0	1,00	2,50	5,00
TOC (mg/L)	24,54	25,39	29,95	40,84	24,69	33,87	42,84	57,53
TC (mg/L)	46,54	46,13	49,07	61,21	44,03	53,37	63,11	77,10
IC (mg/L)	22,01	20,74	19,12	20,36	19,33	19,49	20,27	19,57

Since the purpose of this comparison is to confirm whether the preliminary results could be replicated so optimization could ensue, it is expected that properties such as freeness, app. density and BW register the same values. The same applies to the strength indexes to be compared between trials.

It is clear by looking at Figure 3.23 and Table 3.21 that that is not the case. Freeness increased for both the control and the 1,00 kg/t odp samples. Freeness from the sample treated with a 5,00 kg/t odp ES was on the same level as that of those two samples (Table 3.21). In the repeatability trial, less mass was lost in every sample tested, yet the app. density didn't increase in any case, as seen in Table 3.21).

The discrepancy is more noticeable when analyzing the strength indexes results in Figure 3.22, Figure 3.21 and Figure 3.24. Due to the better result for the control sample on the repetition trial, the steady increase previously described for NS-51180 is no longer verified, seeing as all the ES treated samples underperformed in comparison (Figure 3.22). No significant change was verified among them. The underperformance is also apparent for burst strength index, as seen in Figure 3.24 c). Not only there weren't any significant changes, but all of the samples registered worse results than in the first trial. This means that other factors were at play that, compounded, had a much stronger influence in the physical strength of the handsheets. In any case, it wasn't possible to reproduce the results of the first trial.

Also noteworthy is the fact that as handsheets performed worse in terms of tensile and burst, it performed better on tear, which is expected.

3.3.2 NS-51003

Results regarding the comparison between the first dosage curve and the repeatability trial for NS-51003 are as follows.

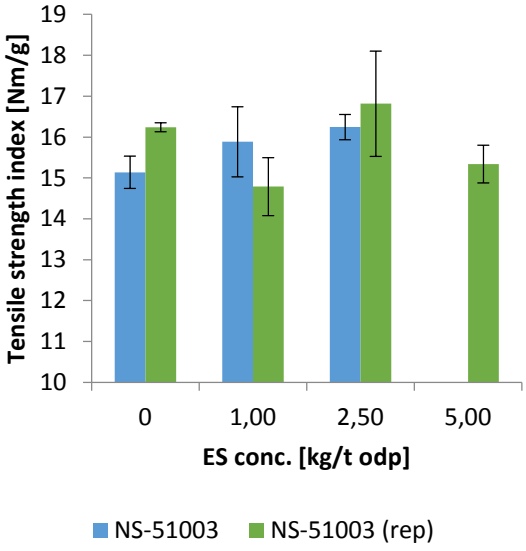

Figure 3.28 – Effect of ES concentration change on tensile strength index for NS-51003 on bleached TMP.

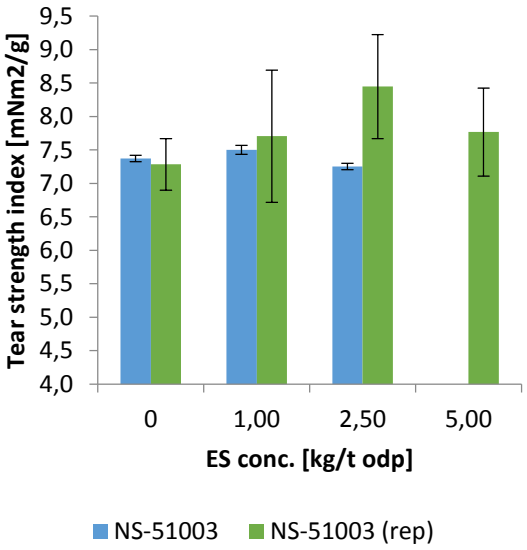

Figure 3.27 – Effect of ES concentration change on tear strength index for NS-51003 on bleached TMP.

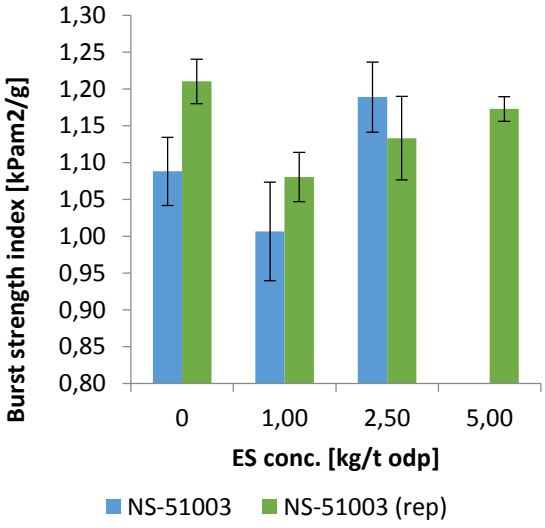

Figure 3.26 – Effect of ES concentration change on burst strength index for NS-51003 on bleached TMP.

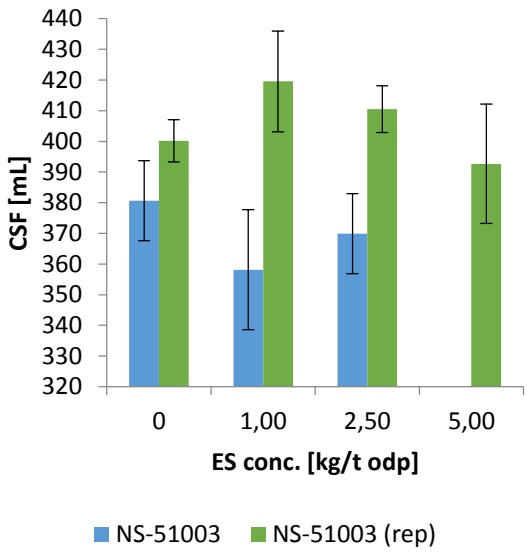

Figure 3.25 – Effect of ES concentration change on freeness for NS-51003 on bleached TMP.

Table 3.24 – Values of the strength indexes and freeness for the control samples and gains for each tested ES concentration for NS-51003 on bleached TMP.

Enzyme	NS-51003				NS-51003 (rep)			
ES conc. (kg/t odp)	0	1,00	2,50	5,00	0	1,00	2,50	5,00
Tensile (Nm/g)	15,14	3,11%	4,93%	7,31%	16,24	-8,95%	3,54%	-5,53%

Tear (mNm^2/g)	7,37	-4,49%	-7,50%	9,28%	7,28	5,80%	15,96%	6,64%
Burst ($kPam^2/g$)	1,09	-4,49%	-7,50%	9,28%	1,21	-10,73%	-6,38%	-3,09%
CSF (mL)	380,6	-2,78%	3,22%	1,63%	400,1	4,85%	2,60%	-1,86%

Table 3.25 – Values of the basis weight, apparent density and ISO brightness for each tested ES concentration for NS-51003 on bleached TMP.

Enzyme	**NS-51003**				**NS-51003 (rep)**			
ES conc. (kg/t odp)	**0**	**1,00**	**2,50**	**5,00**	**0**	**1,00**	**2,50**	**5,00**
BW (g/m^2)	93,82	91,64	88,77	89,21	93,78	87,36	92,84	92,13
App. density (g/cm^3)	0,352	0,358	0,341	0,383	0,359	0,351	0,388	0,343
ISO brightness (%)	58,08	57,35	56,20	56,49	59,33	59,00	58,44	57,95

Table 3.26 – Fiber characteristics for each tested ES concentration for NS-51180 on bleached TMP.

Enzyme	**NS-51003**				**NS-51003 (rep)**			
ES conc. (kg/t odp)	**0**	**1,00**	**2,50**	**5,00**	**0**	**1,00**	**2,50**	**5,00**
Crill	150,52	151,39	150,54	156,40	152,80	152,84	151,92	151,87
Length (mm)	1,92	1,72	1,76	1,61	1,92	1,73	1,87	1,89
Fines (%)	45,4	53,8	56,1	42	46,5	56,7	51,3	44,4
Width (µm)	34,8	34,8	35,5	34,5	35,6	34,4	34,3	34,6
Curl (%)	9,3	9,4	9,6	9,4	9,1	10,2	9,8	8,7
Kinks/mm	0,12	0,11	0,08	0,09	0,10	0,08	0,08	0,09

Table 3.27 – TOC, TC and IC for each tested ES concentration for NS-51003 on bleached TMP.

Enzyme	**NS-51003**				**NS-51003 (rep)**			
ES conc. (kg/t odp)	0	0,50	1,00	2,50	0	1,00	2,50	5,00
TOC (mg/L)	417,9	527,3	49,47	561,3	551,9	416,5	422,5	550,5
TC (mg/L)	418,4	527,7	70,43	561,7	552,40	417,00	422,90	550,90
IC (mg/L)	0,47	0,46	20,96	0,46	0,50	0,43	0,45	0,42

Some of the same problems pointed out for NS-51180 are present in the trials for NS-51003. Better performance of the control samples for both tensile and burst make it so that the trends verified in the first dosage curves are not repeated in the confirmation trials and freeness results are greater in the confirmation trials. The BW in the confirmation trial wasn't greater, though. There was some variability in terms of app. density. Results couldn't be confirmed.

3.4 Control comparison, closing comments and suggestions for future work

Throughout the discussion, there were some instances were expected trends that were not verified. This may be attributed to the substrate itself. The best way to assess this is to compare the control samples for all the different trials.

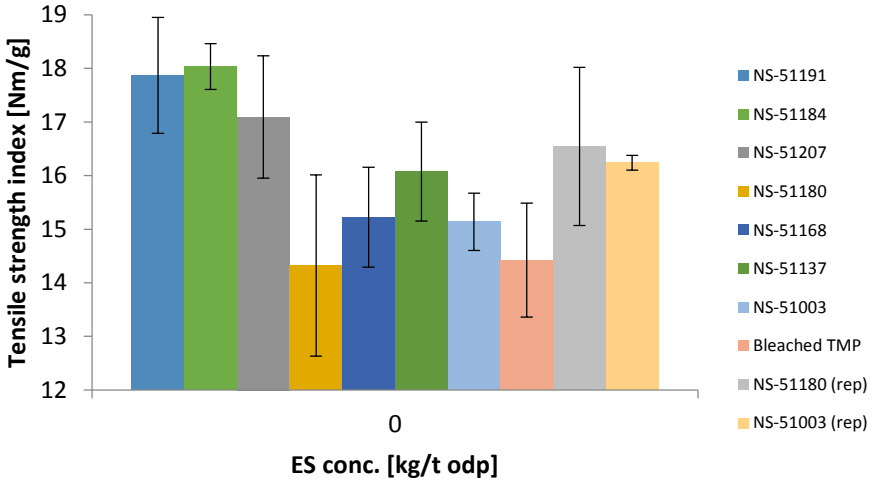

Figure 3.29 Tensile strength index control comparison for all the trials performed with bleached TMP.

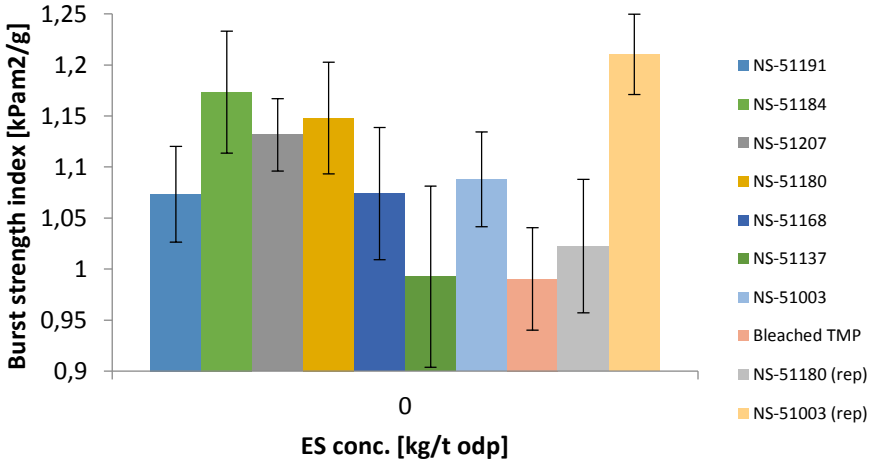

Figure 3.31 – Burst strength index control comparison for all the trials performed with bleached TMP.

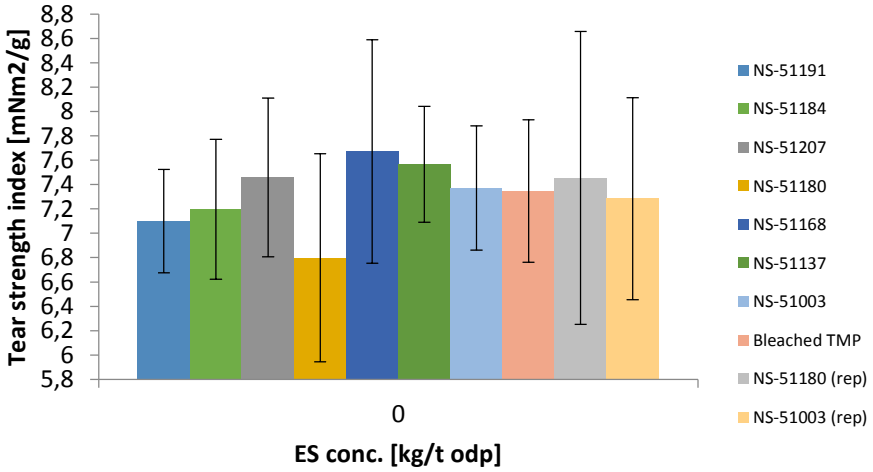

Figure 3.30 – Tear strength index control comparison for all the trials performed with bleached TMP.

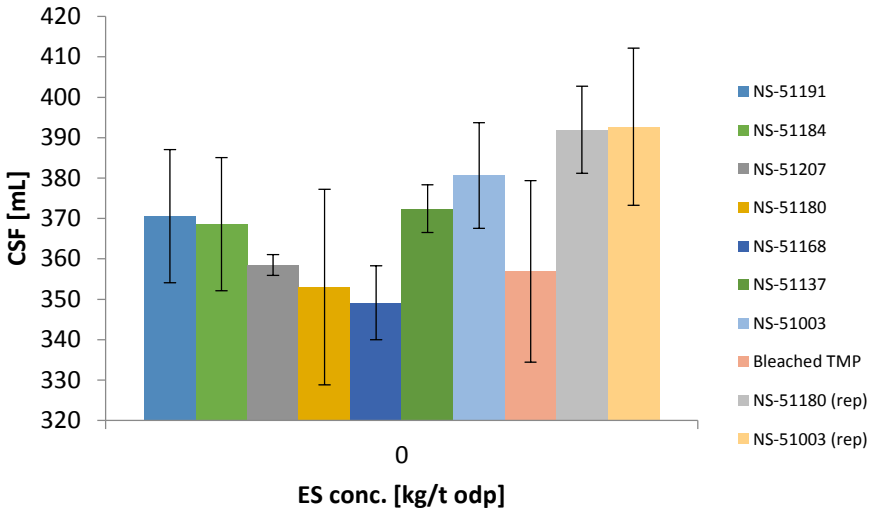

Figure 3.32 – Freeness control comparison for all the trials performed with bleached TMP.

As can be seen in Figure 3.29, Figure 3.31, Figure 3.30 and Figure 3.32, there is significant variability for all of the properties. Being as these are control samples from the same substrate the results should not show this level of variability. This may be due to the fiber length and especially due to the percentage of fines, which ranges from 35 to nearly 60 %. Fibrillation might have also had an influence on the results, although not as significant, since variability in this parameter wasn't as big. What is evident though, is that pulp in general and TMP specifically are multivariate systems and analyzing these type of system requires the implementation of tools that facilitate the manipulation of data concerning these systems.

It is also important to note that the conditions for testing all the enzymes hereby presented, while theoretically adequate, were very uniform and better results may be possible to obtain by testing them at different temperatures, pH, and/or using different buffers and ES concentrations.

Another possibility to be considered is testing laccases thoroughly and optimize the conditions in which they could be the most effective, for they could modify lignin to the extent that it be easier to access the inner layers of the fibers that way. Not only this could potentially have significantly positive effects for physical resistance, but it could enable enzymes such as xylanases, mannanases and cellulases to reach their respective substrates more easily and have significant effects on the fibers and, consequently, on the physical strength of the paper.

Chapter 4 Conclusions

This project aimed to study the effects treatment with enzymes manufactured by Novozymes A/S would have on physical properties of paper manufactured from MP.

For this intent, dosage curves were generated on TMP accepts previously bleached with H_2O_2. These trials were performed after making refining curves and deciding 1000 revolutions as the level of refining to apply in the dosage curves. The classes of enzymes tested were xylanases (NS-51191, NS-51207 and NS-51168), mannanases (NS-51184 and NS-51180), a cellulase (NS-51137) and laccase (NS-51003). Dosage curves for NS-51180 and NS-51003 were repeated seeking to confirm the increases initially observed. Total organic carbon was measured from the filtrates of samples from the aforementioned trials. Fiber characteristics were also measured in the PulpEye.

Pulp is a multivariant system. There are many parameters that influence the outcome when it comes to its physical strength, and that are related to the quality of the fibers themselves or to their ability to bond to one another. Tear strength is usually associated with the former, tensile and bursting with the latter. Factors like fibrillation, percentage of fines and fiber length have a big role in inter-fiber bonding, i.e. tensile and bursting, whereas basis weight and density were shown to relate better to tear. Parameters such as fiber width, curl and kinks/mm remained pretty much constant within the various trials, which means they did not have a significant role in the variations that came up. Two strategies were implemented to try and maximize physical resistance: refining, which is the most common applied to MP, precisely because of benefits like increasing fibrillation and creating fines, even though it may also shorten the fibers; and bleaching with H_2O_2, which acts upon lignin compounds known as chromophores, not only brightening the pulp, but also exposing the inner layers of the fibers more and thus increasing bonding, while keeping the yield loss to a minimum.

Of the enzymes tested, NS-51191, NS-51184, NS-51180, NS-51137 and NS-51003 were considered to have reacted to one extent or another. Of these, NS-51180 and NS-51003 were chosen to be tested again, because the results suggested the strength properties, namely tensile and burst, could show more expressive results if a higher enzyme dose was applied for treatment. The confirmation of those results and expectations did not occur, though. Regardless of this outcome, the changes that occurred weren't much greater than 13%, all the while having tensile values that never

got beyond 20 Nm/g. Also, the samples were characterized for having a lot of variability, as comparison between the control samples showed, making it harder to conclude those occasional improvements by enzymatic treatment were relevant. Thus, the hope that enzymatic treatment would increase physical strength of the pulp significantly was not fulfilled. The testing conditions were very uniform for all the enzymes, only changing the pH, depending on the enzyme at study at any given trial.

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Appendices

Appendix A- Materials and Methods

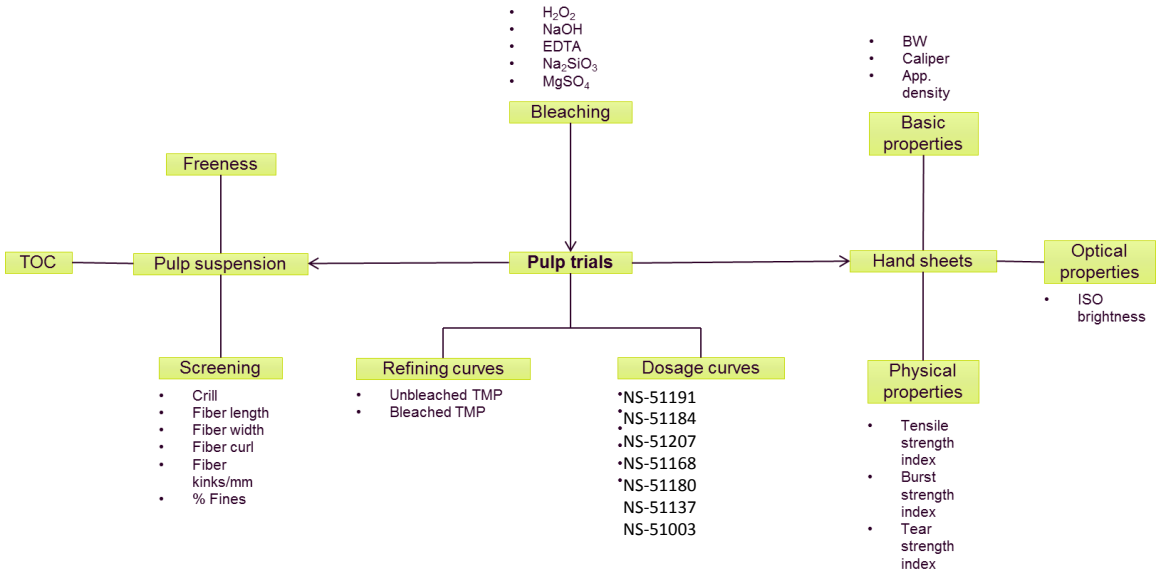


Figure A.1 – Overview of the methods implemented throughout the project.

Guideline table to prepare Britton-Robinson buffers

PREPARATION OF BRITTON-ROBINSON BUFFER (B-R buffer)
SISa/20. Nov. 1995
Updated SISa/23. June 1998
Adapted from method available in EDA, Cellulase Technology, NNAS.

1) Britton-Robinson Buffer can be prepared to cover the range pH 4 to pH 11.6.
2) Mix PART A and PART B in the ratios indicated.
3) Fine adjust the resulting solution to the desired pH by adding a few drops of 1N or 5N NaOH (or use one of the acids in the mix if the pH must be lowered).

			Example: To prepare 1 L each of parts A and B:			
PART A:		mw (g/mol)	Mass (g)	EDAChem	Phys. Form	Purity (%)
0.04 M H3PO4	Orthophosphoric Acid	98.00	4.61	37 H3C	liquid	85
0.04 M CH3COOH	Acetic Acid	60.05	2.40	40 H3C	liquid	100
0.04 M H3BO3	Orthoboric Acid	61.83	2.47	96 CS1	powder	100
			(then dilute to 1 L with de-ionized water)			
PART B:						
0.2 M NaOH	Sodium Hydroxide	40.00	8.00	8 CS1	pellet	100
			(then dilute to 1 L with de-ionized water)			

Combine parts A and B according to the chart below:

pH	To make 5 L		To make 1 L		To make 100 mL	
	Part A (L)	Part B (L)	Part A (L)	Part B (L)	Part A (mL)	Part B (mL)
4	4.03	0.98	0.806	0.196	80.6	19.6
5	3.71	1.29	0.742	0.258	74.2	25.8
6	3.53	1.48	0.706	0.296	70.6	29.6
7	3.28	1.72	0.656	0.344	65.6	34.4
8	3.12	1.88	0.624	0.376	62.4	37.6
9	2.98	2.03	0.596	0.406	59.6	40.6
10	2.81	2.19	0.562	0.438	56.2	43.8
11	2.73	2.27	0.546	0.454	54.6	45.4

More concentrated buffers can be made by increasing the amounts used to prepare parts A and B.

Desired Buffer Strength (M)	Multiply Mass by
0.04	1
0.05	1.25
0.10	2.5
0.50	12.5

Calculation examples

Determination of the concentration of a Britton-Robinson buffer

- To prepare 5 L of a Britton-Robinson buffer, pH = 6

$$C_{BR} = \frac{\frac{V_A}{V_A^{1L}} \times \left(\frac{0{,}85 \times m_{H_3PO_4}}{M_{H_3PO_4}} + \frac{m_{CH_3COOH}}{M_{CH_3COOH}} + \frac{m_{H_3BO_3}}{M_{H_3BO_3}}\right)}{V_{BR}} \tag{6}$$

$$= \frac{\frac{3{,}53\ L}{1\ L} \times \left(\frac{0{,}85 \times 4{,}61\ g}{98{,}00\ g/mol} + \frac{2{,}40\ g}{60{,}05\ g/mol} + \frac{2{,}47\ g}{61{,}83 g/mol}\right)}{5\ L}$$

$$= 0{,}0846\ M = 84{,}6\ mM$$

Determination of the mass of H_2O_2 necessary to bleach 24,0 g odp of pulp

$$m_{H_2O_2}^{bleaching} = 24{,}0\ g \times C_{H_2O_2}^{bleaching} = 24{,}0\ g \times \frac{3{,}00}{100} = 0{,}720\ g \tag{7}$$

$$C_{H_2O_2}^{sample} = \frac{m_{H_2O_2}^{bleaching}}{100} = \frac{0{,}720\ g}{5\ cm^3} = 0{,}144\ g/cm^3$$

$$m_{H_2O_2}^{sol} = C_{H_2O_2}^{sample} \cdot V_{sol} = 0{,}144\ g/cm^3 \times 50\ cm^3 = 7{,}20\ g$$

$$C_{H_2O_2}^{bottle} = \frac{m_{H_2O_2}^{sol}}{m_{H_2O_2}^{needed}} \Leftrightarrow m_{H_2O_2}^{needed} = \frac{m_{H_2O_2}^{sol}}{C_{H_2O_2}^{bottle}} = \frac{7{,}20\ g}{0{,}3000} = 24{,}0\ g$$

Determination of the mass of enzyme necessary to prepare an ES with a specific concentration

- To prepare a 2,50 kg/t odp ES

$$m_{enz}^{2{,}50\ kg/t\ odp} = 24{,}0\ g \times \frac{2500\ g}{10^6\ g} = 0{,}0600\ g = 60{,}0\ mg \tag{8}$$

$$C_{sample}^{2{,}50\ kg/t\ odp} = \frac{m_{enz}^{2{,}50\ kg/t\ odp}}{V_{sample}} = \frac{60{,}0\ mg}{10{,}0\ cm^3} = 6{,}00\ mg/cm^3$$

$$m_{enz}^{sample} = C_{sample} \cdot V_{sol} = 6{,}00 \frac{mg}{cm^3} \times 100{,}0\ cm^3 = 600\ mg$$
$$= 0{,}600\ g$$

- To prepare a 1,00 kg/t odp ES

$$f = \frac{C_{sample}^{2{,}50\ kg/t\ odp}}{C_{sample}^{1{,}00\ kg/t\ odp}} = \frac{6{,}00\ mg/cm^3}{2{,}40\ mg/cm^3} = 2{,}50 \tag{9}$$

$$f = \frac{V_{sol}^{1{,}00\ kg/t\ odp}}{V_{sol}^{2{,}50\ kg/t\ odp}} \Leftrightarrow V_{sol}^{2{,}50\ kg/t\ odp} = \frac{V_{sol}^{1{,}00\ kg/t\ odp}}{f}$$

$$V_{sol}^{2{,}50\ kg/t\ odp} = \frac{25{,}0\ cm^3}{2{,}50} = 10{,}0\ cm^3$$

Determination of the pulp necessary to have 24,0 g odp

$$m_{pulp}^{T} = \frac{24{,}0\ g}{\%\ DC} \tag{10}$$

$$= \frac{24{,}0\ g}{0{,}5184}$$

$$= 46{,}3\ g$$

Determination of the mass of a pulp suspension with 2% consistency

$$m_{susp} = \frac{24{,}0\ g}{Consistency} \tag{11}$$

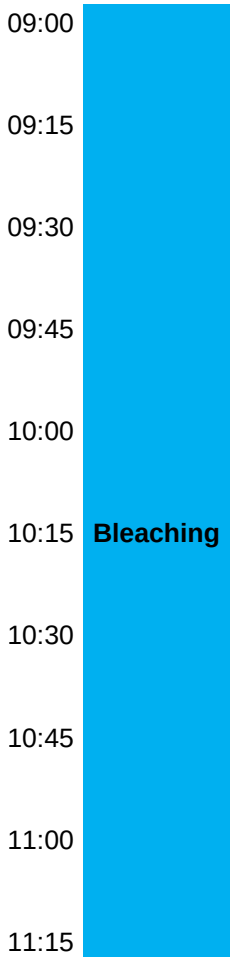
$$= \frac{24{,}0\ g}{0{,}02}$$

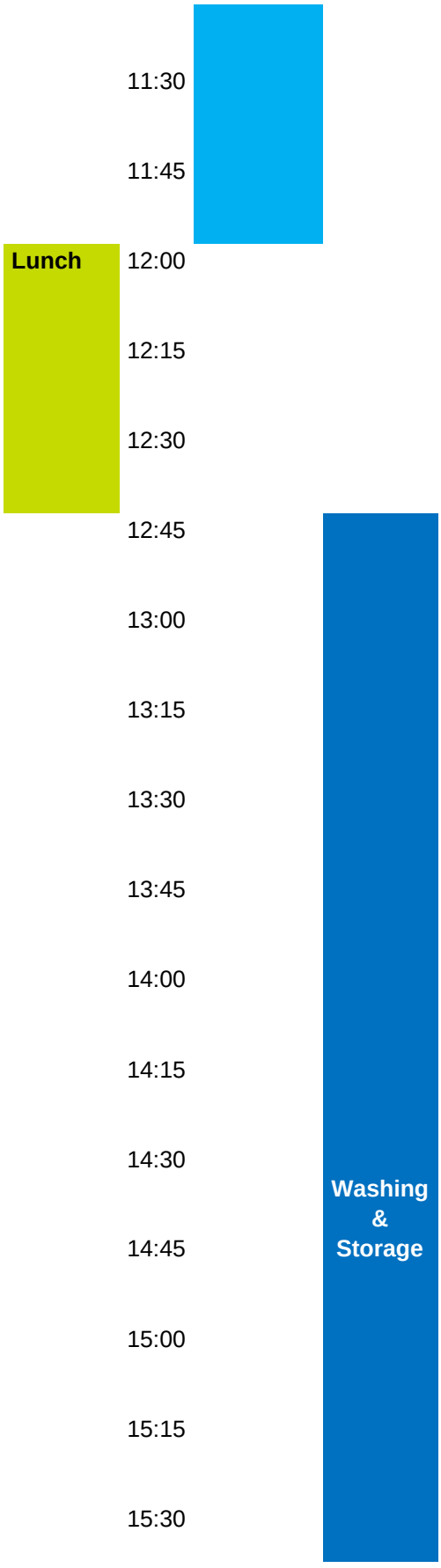
$$= 1200{,}0\ g$$

Schedule for bleaching with H_2O_2

Table A.1 – Guideline timetable for handling all the samples to be bleached in a single day

Sample	Pre-heating (min)	NaOH addition (min)	Out of the water bath (min)
1	0	5	65
2	5	10	70
3	10	15	75
4	15	20	80
5	20	25	85
6	25	30	90
7	30	35	95
8	35	40	100
9	40	45	105
10	45	50	110

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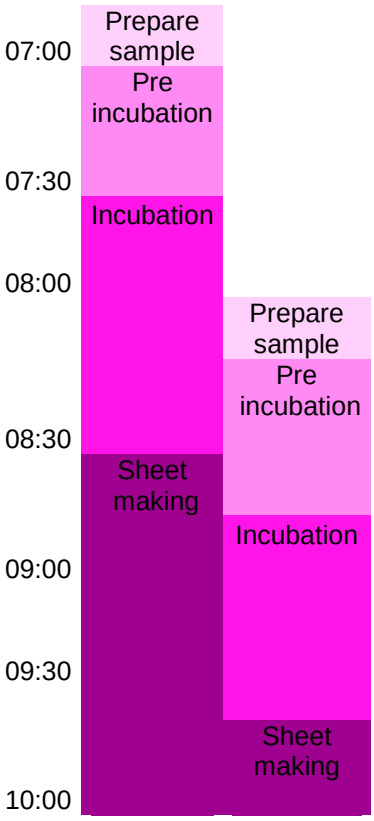
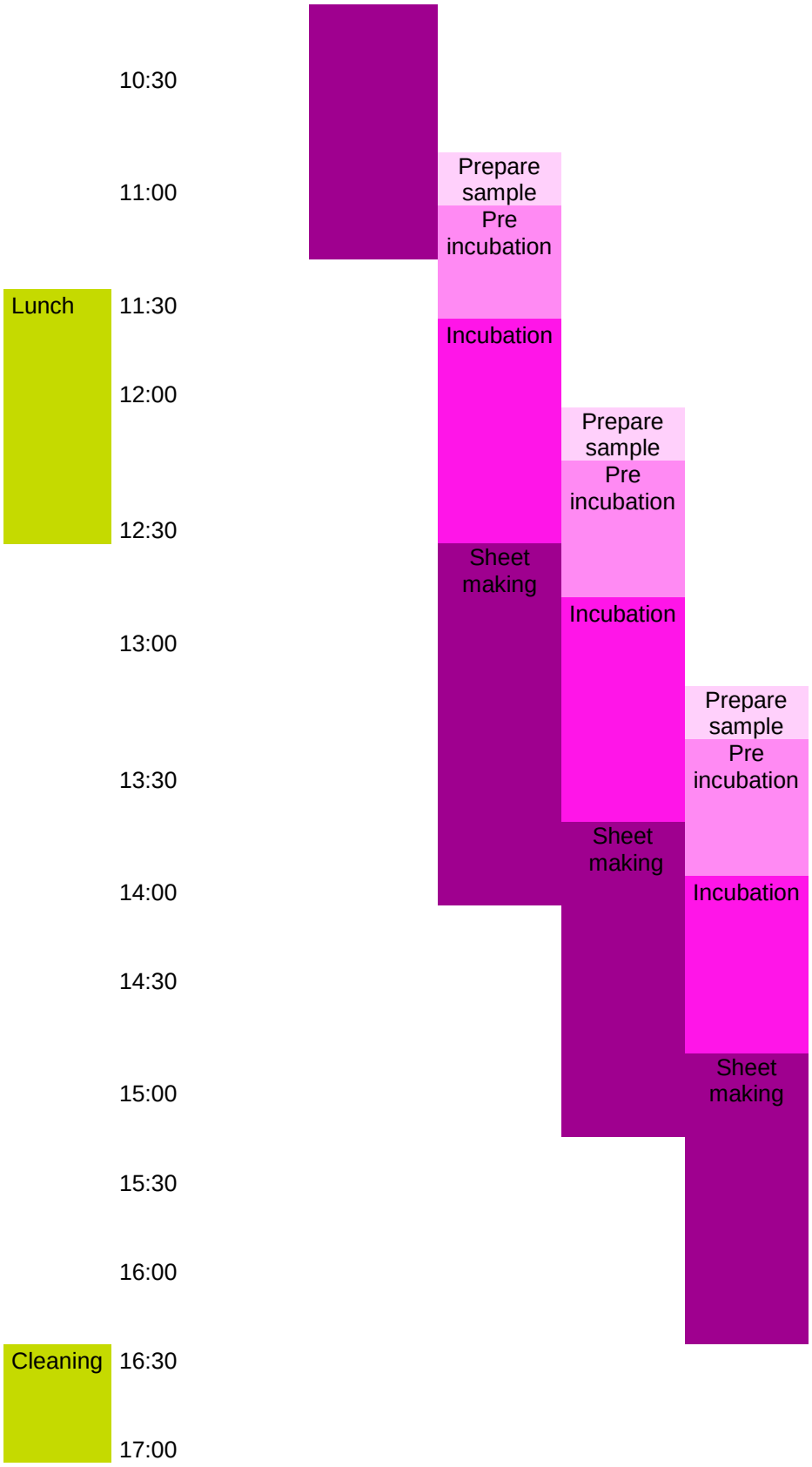
11:45
Lunch
12:00
12:15
12:30
12:45
13:00
13:15
13:30
13:45
14:00
14:15
14:30
Washing
&
Storage
14:45
15:00
15:15
15:30

Schedule for handsheet preparation

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11:00
11:30
12:00
12:30
13:00
13:30
14:00
14:30
15:00
15:30
16:00
16:30
17:00
Lunch
Cleaning
Prepare sample
Pre incubation
Incubation
Sheet making
Prepare sample
Pre incubation
Incubation
Sheet making
Prepare sample
Pre incubation
Incubation
Sheet making

UV-Vis Spectroscopy

Ultraviolet and visible (UV-Vis) molecular spectroscopy is a laboratory technique that makes use of the ultraviolet and visible regions of the electromagnetic spectrum to analyze samples for molecular compounds and complex ions. UV-Vis spectroscopy is used to identify unknown species and detecting impurities in known species by comparing the absorption or transmission spectra with known spectra, or to quantify the concentration of a given species by using the Beer-Lambert's law

$$A = \varepsilon bc \tag{12}$$

where A corresponds to the absorbance, b to the pathlength and c to the concentration.

The UV-Vis spectroscopy is sensitive to wavelengths between 190 and 900 nm. The human eye is sensitive to light with wavelengths from 400 to 750 nm. [22], [23]

Absorbance measurements were made on a UV-Vis spectrophotometer at 590 nm making use of a standard compound for both xylanases and mannanases. The intent was to assess its reactivity by changing the ES concentration. In the cases where there was a favorable response, one of the tested ES concentrations would be singled out and the reactivity for pH would be tested. The same for temperature changes. Once the best settings were gotten, they would be tested with the TMP samples.

This method was not included on the main body of the report, since the it was later decided the samples would be treated with different concentrations from a given enzyme. Not all the enzymes tested to that point were responding well to this method.

Appendix B- Results and Discussion

Calculation examples

Determination of BW

$$BW = \frac{Total\ weight\ of\ sheets \times 50}{Number\ of\ sheets} \tag{13}$$

$$= \frac{9{,}0877 \times 50}{5} = 90{,}88\ g/m^2$$

Determination of app. density

$$App.density = \frac{BW}{1000 \times Caliper} \tag{14}$$

$$= \frac{90{,}88}{1000 \times 0{,}26328} = 0{,}345\ g/cm^3$$

Determination of tensile strength index

$$Tensile\ strength\ index = \frac{Tensile\ strength}{BW} \tag{15}$$

$$= \frac{1535{,}1}{91{,}67} = 16{,}75\ N \cdot m/g$$

Determination of burst strength index

$$Burst\ strength\ index = \frac{Burst\ strength}{BW} \tag{16}$$

$$= \frac{6{,}89476 \times 14{,}2}{91{,}67} = 1{,}07\ kPa \cdot m^2/g$$

Determination of tear strength index

$$Tear\ strength\ index = \frac{Tear\ strength}{BW} \tag{17}$$

$$= \frac{61{,}4 \times 10}{91{,}67} = 6{,}76\ mN \cdot m^2/g$$